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EVALUATION OF 10 PESTICIDE METHODS

by

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16. ABSTRACT

Ten pesticide analysis methods were evaluated. The compounds listed in each method were analyzed in triplicate at two concentration levels in reagent water and POTW effluent. Each method was performed as written with only minor modifications as approved by the USEPA Project Officer. If a cleanup procedure was included in the analysis method, all analyses were performed with and without the cleanup step.

Resultant data reported included estimated detection limits (EDLs) in reagent water and recovery data from reagent water and POTW effluent for each compound. Suggestions for method improvements were included in the report where necessary.

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FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring and Support Laboratory-Cincinnati conducts research to:

- Develop and evaluate methods to measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid waste.

- Investigate methods for the concentration, recovery, and identification of viruses, bacteria and other microbiological organisms in water; and, to determine the responses of aquatic organisms to water quality.

- Develop and operate an Agency-wide quality assurance program to assure standardization and quality control of systems for monitoring water and wastewater.

- Develop and operate a computerized system for instrument automation leading to improved data collection, analysis, and quality control.

This report describes the testing and evaluation of ten pesticide analysis methods to be proposed for the analysis of selected pesticides in wastewater.

Robert L. Booth, Director
Environmental Monitoring and Support
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ABSTRACT

Ten pesticide analysis methods were evaluated. The compounds listed in each method were analyzed in triplicate at two concentration levels in reagent water and POTW effluent. Each method was performed as written with only minor modifications as approved by the EPA Project Officer. If a cleanup procedure was included in the analysis method, all analyses were performed with and without the cleanup step.

Resultant data reported included estimated detection limits (EDLs) in reagent water and recovery data from reagent water and POTW effluent for each compound. A summary of the resultant data is given in Table 1. Suggestions for method improvements are included in the report where necessary.

TABLE 1. SUMMARY OF DATA FROM EVALUATION OF 10 PESTICIDE ANALYSIS METHODS

Method Number	Compound	EDL, μg/L	Spike Level, μg/L		Recovery from Reagent Water, %[d]				Recovery from POTW Effluent[d]			
					Before Cleanup		After Cleanup		Before Cleanup		After Cleanup	
			Low	High	Low	High	Low	High	Low	High	Low	High
641	Thiabendazole	1.7	10	100	93 ± 3	81 ± 5	N.C.[a]	N.C.	96 ± 5	100 ± 2	N.C.	N.C.
641.1	Ethoxyquin	6.3	6.2	62	100 ± 32	82 ± 6	N.C.	N.C.	19 ± 14	58 ± 1	N.C.	N.C.
642	Biphenyl	0.04	2.5	25	74 ± 2	51 ± 11	N.C.	N.C.	56 ± 4	61 ± 1	N.C.	N.C.
642	O-Phenylphenol	0.01	5.0	50	73 ± 4	60 ± 5	N.C.	N.C.	69 ± 2	82 ± 15	N.C.	N.C.
643	Bentazon	1.1	10	100	92 ± 2	81 ± 10	N.C.	N.C.	94 ± 7	76 ± 2	N.C.	N.C.
632.1	Carbaryl	1.2	2.0	20	52 ± 10	83 ± 13	N.C.	N.C.	N.D.	28 ± 16	N.C.	N.C.
632.1	Napropamide	0.02	6.0	60	103 ± 2	102 ± 2	N.C.	N.C.	96 ± 7	94 ± 3	N.C.	N.C.
632.1	Propanil	0.3	0.2	2	79 ± 6	99 ± 3	N.C.	N.C.	85 ± 11	77 ± 7	N.C.	N.C.
644	Picloram	0.3	3.0	30	N.D.	N.D.	N.D.	N.D.	52 ± 9	71 ± 0	58 ± 4	68 ± 3
614.1	Dioxathion	0.5	10	100	76 ± 10	78 ± 2	43 ± 7	58 ± 6	87 ± 15	91 ± 3	67 ± 6	74 ± 3
614.1	EPN	12	10	100	120 ± 6	120 ± 4	N.D.	65 ± 14	85 ± 2	110 ± 6	N.D.	62 ± 4
614.1	Ethion	0.3	10	100	120 ± 5	95 ± 2	54 ± 8	78 ± 11	94 ± 5	86 ± 5	59 ± 12	79 ± 3
614.1	Terbufos	0.02	10	100	90 ± 3	84 ± 1	N.D.	42 ± 13	94 ± 5	77 ± 3	57 ± 4	54 ± 8
645	Alachlor	0.2	10	100	96 ± 3	94 ± 2	96 ± 3	94 ± 3	109 ± 1	102 ± 1	105 ± 3	97 ± 3
645	Butachlor	0.2	10	100	96 ± 4	93 ± 1	95 ± 3	93 ± 2	103 ± 1	100 ± 1	104 ± 5	95 ± 2
645	Diphenamid	0.2	10	100	93 ± 6	94 ± 2	95 ± 2	97 ± 3	105 ± 1	103 ± 1	95 ± 3	94 ± 4
645	Lethane	0.1	10	100	93 ± 6	100 ± 1	97 ± 2	99 ± 4	120 ± 2	123 ± 1	108 ± 4	106 ± 3
645	Norflurazon	0.02	10	100	69 ± 10	92 ± 1	69 ± 6	60 ± 6	107 ± 1	108 ± 2	76 ± 11	65 ± 17
645	Fluridone	0.6	10	100	49 ± 16	81 ± 15	N.C.	N.C.	124 ± 6	111 ± 2	N.C.	N.C.
646	Basalin	0.0005	0.1	1	138 ± 12	113 ± 4	126 ± 8	109 ± 10	94 ± 6	113 ± 2	74 ± 3	108 ± 3
646	CDNB	0.0005	0.1	1	91 ± 8	69 ± 5	89 ± 6	71 ± 5	78 ± 2	71 ± 2	76 ± 4	70 ± 2
646	Dinocap	0.1	0.1	1	78 ± 7	77 ± 4	26 ± 3	72 ± 14	76 ± 40	72 ± 3	123 ± 53	80 ± 10
608.2	Chlorothalonil	0.001	0.02	0.2	I[c]	I	I	I	I	I	I	I
608.2	DCPA	0.003	0.02	0.2	128 ± 18	94 ± 11	73 ± 25	62 ± 5	N.D.	62 ± 5	71 ± 5	76 ± 2
608.2	Dicloran	0.002	0.01	0.1	I	I	I	I	I	I	I	I
608.2	Methoxychlor	0.04	0.1	1	57 ± 1	106 ± 12	37 ± 8	86 ± 12	47 ± 12	99 ± 2	40 ± 27	90 ± 14
608.2	Cis-permethrin	0.2	1.0	10	94 ± 9	91 ± 2	91 ± 6	128 ± 17	89 ± 8	85 ± 3	97 ± 14	98 ± 27
608.2	Trans-permethrin	0.2	1.0	10	111 ± 4	98 ± 3	83 ± 12	108 ± 13	85 ± 2	90 ± 2	41 ± 11	95 ± 29

(a) N.C. = No cleanup procedure included in this method.
(b) N.D. = Not detected.
(c) I = Presence of interferences precluded determination of compound in sample.
(d) Standard deviation is included.

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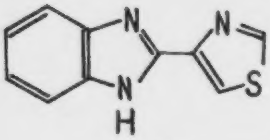
SECTION 1

ANALYSIS OF THIABENDAZOLE IN WASTEWATER BY LIQUID CHROMATOGRAPHY

INTRODUCTION

Thiabendazole (I) is used as a fungicide on various fruits and vegetables. The IUPAC name for thiabendazole is 2-(thiazol-4-yl)benzimidazole and its CAS registry number is 148-79-8. Common synonyms for thiabendazole include TBZ, Mertect, Tecto, Storite, MC-360, Thiaben, Thibenzole, Bovizole, Eprofile, Equizole, Omniqole, and Mintezol. Thiabendazole is a colorless powder with a melting point of 304-305°C; thiabendazole sublimes when heated to 310°C. Thiabendazole is stable under normal conditions to hydrolysis, light, and heat. The solubility of thiabendazole is 10 g/L at 25°C and pH 2. Thiabendazole becomes markedly less soluble in water as the pH is raised. The acute oral LD50 in rats for thiabendazole is 3300 mg/kg (1).



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Thiabendazole was originally determined in crops by fluorometry after extraction with various solvents (2). Several gas chromatographic (GC) methods have been reported (3-5). These procedures require derivatization of the thiabendazole prior to GC analysis. Various high performance liquid chromatographic (HPLC) methods have also been reported (6-14,19). These methods are ultraviolet (UV) (7,9,10,12,13) and fluorescence (6,8,11-14,19) and normal-phase columns (6,7,9,13). In some cases, base or ion-pairing agents are added to the HPLC mobile phase to improve thiabendazole peak shape (7-10,14). Most of the reported methods described the determination of thiabendazole in crops or body fluids. One method described the determination of thiabendazole in wastewaters (14). This procedure is identical to the procedure evaluated in this study. Cleanup methods were not reported for any of these procedures with the exception of minimal acid/base partitioning procedures.

The method provided by the Project Officer for evaluation for determination of thiabendazole in wastewaters consisted of acidification of the sample to solubilize the thiabendazole; filtration of the sample to remove particulate matter; readjustment of the sample pH to make it more compatible with HPLC conditions; and analysis of the sample by HPLC using a fluorescence detector.

CONCLUSIONS

An analysis method designed to determine thiabendazole in wastewaters was found to be acceptable with minor modifications as discussed in the Recommendations section. The method was applied to reagent water and Columbus POTW effluent samples spiked at the 10 or 100 µg/L levels with thiabendazole. Recoveries of thiabendazole from all samples were greater than 81 percent, indicating that the filtration step used to remove particulate from the sample did not cause losses of thiabendazole. The HPLC-fluorescence conditions used for sample analyses yielded acceptable chromatography, sensitivity, and selectivity.

EXPERIMENTAL

The following procedure was outlined in the thiabendazole analysis method:

1. Filter the acidified sample through a 0.45 micron Nylon filter

2. Adjust the pH of the sample to within the range of 7-9 with diluted sodium hydroxide or sulfuric acid

3. Analyze the sample by HPLC with fluorescence detection using the following conditions:
   - 10-micron reverse-phase Ultrasphere ODS, 4.6 mm by 250 mm column;
   - isocratic 70 percent methanol/30 percent buffer (pH 8.2) mobile phase;
   - flow rate of 1 mL/min;
   - injection volume of 100 µL
   - excitation and emission wavelengths of 300 nm (5-nm slit width) and 360 nm (10-nm slit width), respectively.

Prior to performance of the analysis method, two modifications were made in the method after consultation with the EPA Task Officer:

1. A 10-micron reverse-phase Ultrasphere ODS column was not commercially available, so a 5-micron reverse-phase Ultrasphere ODS column was substituted.

2. Although an initial pH adjustment step is mentioned in Section 2 of the method, no such step appears in Section 10.1, Sample Preparation. This initial pH adjustment was included to solubilize the thiabendazole in the sample prior to filtration of the sample. The samples were adjusted to a pH range of 1.0 to 3.0 with

dilute sodium hydroxide or dilute sulfuric acid prior to filtration as described in Section 7.2 of the method.

Samples processed included the following:

1. Triplicate reagent water samples. Reagent water was obtained from a Millipore system.
2. Triplicate reagent water samples spiked with thiabendazole at the 10 μg/L concentration level.
3. Triplicate reagent water samples spiked with thiabendazole at the 100 μg/L concentration level.
4. Triplicate POTW secondary effluent samples. POTW secondary effluent was obtained from the City of Columbus.
5. Triplicate POTW secondary effluent samples spiked with thiabendazole at the 10 μg/L concentration level.
6. Triplicate POTW secondary effluent samples spiked with thiabendazole at the 100 μg/L concentration level.

Recoveries were determined by comparison to standard solutions of thiabendazole prepared at the 10, 50, and 150 μg/L concentration levels in HPLC mobile phase. Response factors were calculated for the standards and used to determine concentration levels in the abovementioned reagent water and POTW effluent samples.

The EDL for thiabendazole, defined as the concentration of thiabendazole in a sample yielding a signal-to-noise ratio (S/N) of 5, was determined by injecting standard solutions of thiabendazole prepared in HPLC mobile phase. The concentrations of these solutions were in the 1 to 10 μg/L range.

QUALITY ASSURANCE

Instrumentation was set up as described in the method. Sensitivity achieved was approximately equivalent to that reported in the method. Calibration standards consisted of thiabendazole solutions prepared at the 10, 50, and 100 μg/L concentration levels in HPLC mobile phase. Each calibration standard was analyzed in duplicate prior to analysis of any evaluation samples. A selected calibration standard was then analyzed after every five evaluation standards. Response factors were calculated for each calibration run by dividing the concentration level in μg/L by the thiabendazole peak area. Resultant calibration data are reported in Table 2. Response factors were reproducible over the entire calibration range. A response factor of 1.64 was used for calculations of thiabendazole concentrations in the evaluation samples.

TABLE 2. THIABENDAZOLE CALIBRATION DATA

Concentration µg/L	Average Peak Area	Standard Deviation, %	Number of Replicates	Average Response Factor
10.0	5.9	13.	3	1.69
50.0	31.3	2.	3	1.60
150.0	91.6	4.	4	1.64

Standards of thiabendazole were obtained from two independent sources. Thiabendazole from the reference standards repository of the EPA Health Effects Research Laboratory (EPA-HERL) was used for preparation of calibration standards as well as for preparation of the evaluation samples. A test calibration standard was also prepared at the 50 µg/L concentration level from thiabendazole from a chemical supply house. The peak area resulting from the analysis of the test calibration standard was 9.0 percent lower than the average peak area obtained from analyses of the EPA-HERL calibration standard. This indicates that both standards obtained were probably within 10 percent of their specified purities.

RESULTS AND DISCUSSION

Thiabendazole was not detected in unspiked reagent water or POTW effluent samples. The chromatograms obtained from reagent water and POTW effluent blanks did not contain any other peaks resulting from interferences. This was presumably due to the use of fluorescence detection instead of UV detection. Recoveries of thiabendazole from reagent water and POTW effluent were greater than 81 percent. Thiabendazole recovery data are given in Table 3. Examples of chromatograms obtained from the analyses of aliquots of POTW effluent unspiked and spiked with thiabendazole at the 10 µg/L level are shown in Figure 1. Recoveries were reproducible with standard deviations of the percent recovery data being lower than 5.3

The EDL for thiabendazole was determined by analyzing a 2.5 µg/L standard solution of thiabendazole in HPLC mobile phase; the resultant chromatogram is shown in Figure 2. This solution yielded a S/N equal to 7.3. The EDL was calculated to be 1.7 µg/L of thiabendazole in a water sample, which is approximately equal to the EDL of 1.0 µg/L reported in the method. The EDL does not take into account any interferences or recovery losses that might be encountered from a particular matrix.

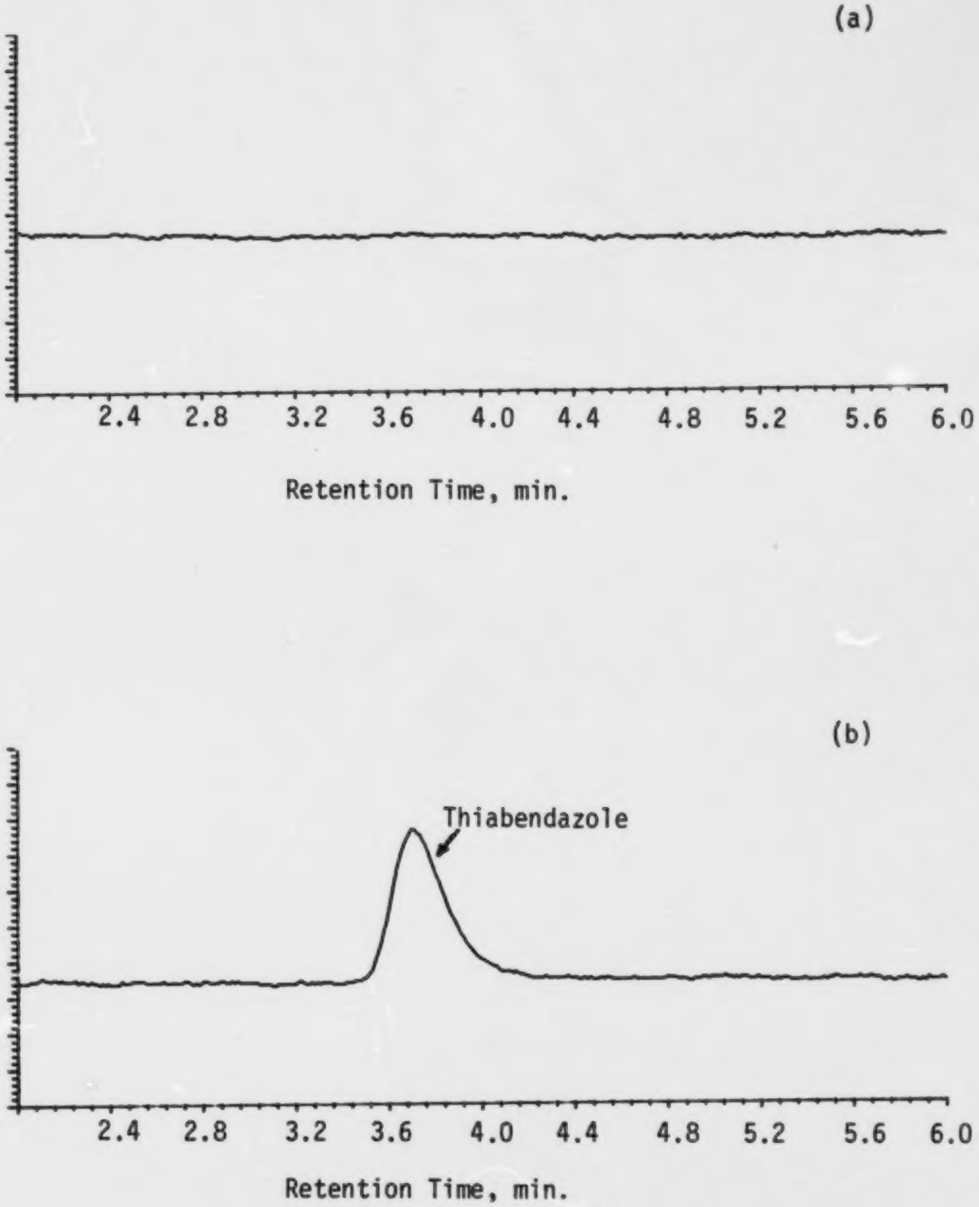


FIGURE 1. HPLC-FLUORESCENCE CHROMATOGRAMS OF (a) POTW EFFLUENT, AND (b) POTW EFFLUENT SPIKED WITH THIABENDAZOLE AT THE 10 μg/L LEVEL.

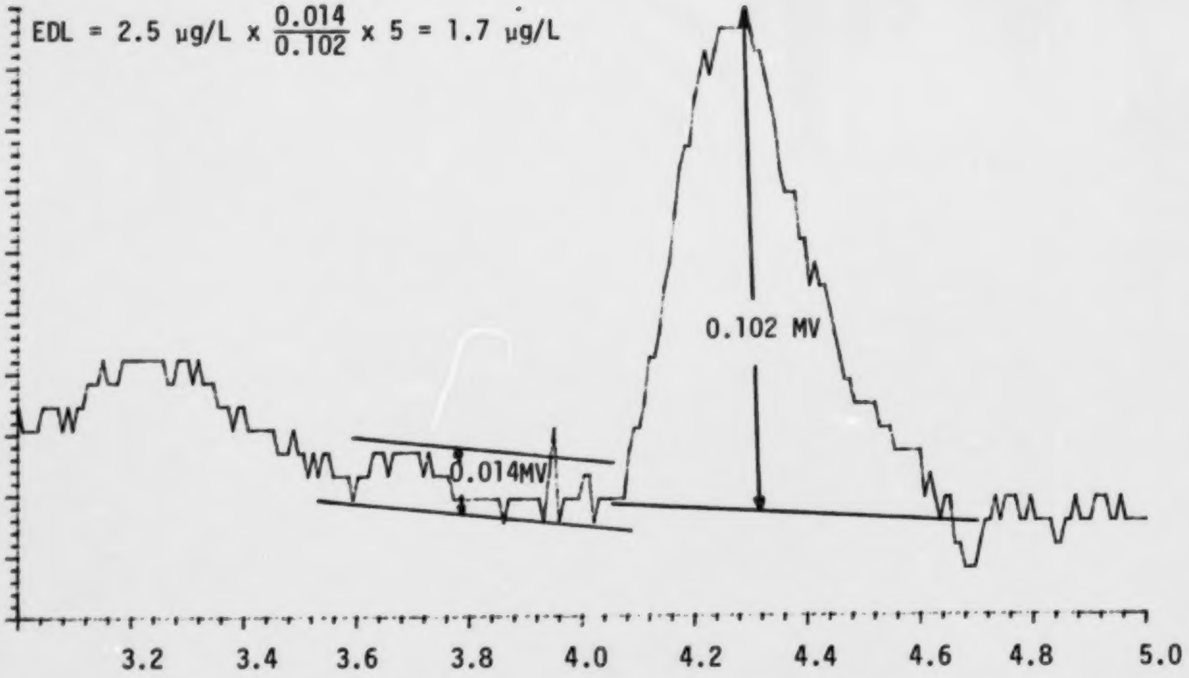


FIGURE 2. HPLC-FLUORESCENCE CHROMATOGRAM OF THIABENDAZOLE STANDARD EQUIVALENT TO 2.5 µg/L IN WATER USED TO DETERMINE METHOD EDL.

TABLE 3. RECOVERIES OF THIABENDAZOLE FROM WATER

Spike Level, µg/L	Matrix[a]	Blank Level µg/L	Percent Recovery[c]	Standard Deviation ,%
10	1	ND[b]	93	3
100	1	ND	81	5
10	2	ND	96	5
100	2	ND	100	2

(a) 1=Reagent water; 2=Columbus POTW secondary effluent.
(b) ND=not detected.
(c) Recovery data are averages of three replicate analyses.

RECOMMENDATIONS

The method was acceptable for determination of thiabendazole in reagent water and POTW effluents. Samples with exceptionally high particulate content may not yield acceptable recoveries of thiabendazole. It is possible that the thiabendazole may adsorb to the particulate matter and be removed with the particulate matter during the filtration step. Although the pH adjustment step prior to filtration may minimize adsorption of the thiabendazole to particulate matter by making it more soluble in the aqueous media, it must be recognized that this may be a serious limitation of the method. It is recommended that this method be studied using wastewaters with high content of particulate matter, such as sludge.

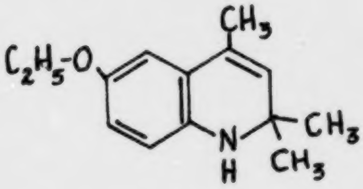
Some difficulty was encountered while using the method when the sample pH was adjusted to 7-9 after filtration. No mention is made in the method that the change in volume due to this pH adjustment must be monitored in order to more accurately determine thiabendazole concentrations in the sample. Volume changes were taken into account when the thiabendazole recovery data presented in Table 2 were calculated. Instructions to measure the volume change due to the final pH adjustment step should be included in the method. Alternatively, this final pH adjustment might not be necessary. The introduction of 100 µL of sample at pH 1-3 may not detectably affect the resultant chromatography.

SECTION 2

ANALYSIS OF ETHOXYQUIN IN WASTEWATER BY LIQUID CHROMATOGRAPHY

INTRODUCTION

Ethoxyquin (II) is used as an antioxidant in animal feeds and food products. The IUPAC name for ethoxyquin is 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline and its CAS registry number is 91-53-2. A synonym for ethoxyquin is Stop-Scald . Ethoxyquin is not reported to be sensitive to light, heat, or pH conditions. Residual chlorine has been reported to degrade ethoxyquin (14). The acute oral LD50 in rats for ethoxyquin is 800-1000 mg/kg.



II

A GC analysis method has been reported for underivatized ethoxyquin in crops using a flame ionization detector (FID) or mass spectrometer (MS) for quantification (15). Close scrutinization of this method indicates non-linearity of the resultant calibration curve at ethoxyquin concentrations less than 500 μg/mL. This is presumably due to adsorption of the amine on the GC column. An alternative GC method using an electron capture detector (ECD) for quantification has also been reported, however this method requires derivatization of the ethoxyquin with heptafluorobutyric anhydride (16). Various HPLC analysis methods have also been reported (14,17-21). These methods use UV (18), fluorescence (14,17,19,21), and electrochemical (20) detection and reverse-phase columns. Most of the reported methods describe the determination of ethoxyquin in crops. One method describes the determination of ethoxyquin in wastewaters (14). This procedure is identical to the procedure evaluated in this study. An adsorption chromatography cleanup procedure using aluminum oxide is reported (17).

The method provided by the Project Officer for evaluation for determination of ethoxyquin in wastewaters consisted of filtration of the sample to remove particulate matter and analysis of the sample by HPLC using a fluorescence detector.

CONCLUSIONS

An analysis method designed to determine ethoxyquin in wastewaters was not acceptable. The method was applied to reagent water and Columbus POTW effluent samples spiked at the 10 or 100 µg/L levels with ethoxyquin. Recoveries of ethoxyquin from POTW effluent were markedly lower than those obtained from reagent water. It is likely that the ethoxyquin was adsorbed onto particulate matter in the samples and consequently removed during filtration of the sample. The HPLC-fluorescence conditions used for sample analyses yielded acceptable chromatography and selectivity. System sensitivity for ethoxyquin was approximately six times that reported in the method, perhaps due to the use of an excitation wavelength of 385 nm instead of the maximum excitation wavelength of 358 nm given in the literature (1).

EXPERIMENTAL

The following procedure was outlined in the ethoxyquin analysis method:

1. Filter the sample through a 0.45 micron Nylon filter.

2. Analyze the sample by HPLC with fluorescence detection using the following conditions:
   - 5-micron reverse-phase Ultrasphere ODS, 4.6 mm by 250 mm column;
   - isocratic 80 percent methanol/20 percent buffer (pH 8.2) mobile phase;
   - flow rate of 1 mL/min;
   - injection volume of 100 µL;
   - excitation and emission wavelengths of 385 nm (5-nm slit width) and 450 nm (10-nm slit width), respectively.

Prior to performance of the analysis method, one modification was made in the method after consultation with the EPA Task Officer. The method states in Section 2 that the "neutral sample" is filtered and then analyzed by direct aqueous injection. However, no pH adjustment step is described in Section 10. The samples were adjusted to pH range 6.5 to 7.5 with dilute sodium hydroxide or dilute sulfuric acid prior to filtration.

Samples processed included the following:

1. Triplicate reagent water samples. Reagent water was obtained from a Millipore system

2. Triplicate reagent water samples spiked with ethoxyquin at the 10 µg/L concentration level*

3. Triplicate reagent water samples spiked with ethoxyquin at the 100 µg/L concentration level*

---

* The standard used for preparation of the calibration standards and the evaluation standards was not as pure as specified by the supplier; all abovementioned samples were spiked at 64 percent of the specified levels.

4. Triplicate POTW secondary effluent samples. POTW secondary effluent was obtained from the City of Columbus.

5. Triplicate POTW secondary effluent samples spiked with ethoxyquin at the 10 μg/L concentration level*

6. Triplicate POTW secondary effluent samples spiked with ethoxyquin at the 100 μg/L concentration level*.

Recoveries were determined by comparison to standard solutions of ethoxyquin prepared at the 10, 50, and 150 μg/L concentration levels in HPLC mobile phase*. Response factors were calculated for the standards and used to determine concentration levels in the abovementioned reagent water and POTW effluent samples.

The EDL for ethoxyquin, defined as the concentration of ethoxyquin in a sample yielding a S/N of 5, was determined by injecting standard solutions of ethoxyquin prepared in HPLC mobile phase. The concentrations of these solutions were in the 1 to 10 μg/L range.

QUALITY ASSURANCE

Instrumentation was set up as described in the method. Sensitivity achieved was slightly lower than that reported in the method. Calibration standards consisted of ethoxyquin solutions prepared at HPLC mobile phase concentrations of 6.3, 32, and 95 μg/L. Each calibration standard was analyzed in duplicate prior to analysis of any evaluation samples. A selected calibration standard was then analyzed after every five evaluation standards. Response factors were calculated for each calibration run by dividing the concentration level in μg/L by the resultant ethoxyquin peak area. Resultant calibration data are reported in Table 4. Response factors were repeatable over the entire calibration. A response factor of 1.42 was used for calculations of ethoxyquin concentrations in the evaluation samples.

Standards of ethoxyquin were obtained from two independent sources. Ethoxyquin from the reference standards repository of EPA-HERL was used for preparation of calibration standards as well as for preparation of the evaluation samples. A test calibration standard was also prepared at the 50 μg/L concentration level from ethoxyquin from a chemical supply house. The peak area resulting from the analyses of the EPA-HERL calibration standards was 64 percent lower than the peak area obtained from analysis of the test calibration standard. The assumption was made that the purity specified by EPA-HERL was incorrect. It was therefore assumed that the calibration standards did not contain ethoxyquin at the 10, 50, and 150 μg/L levels, but actually contained ethoxyquin at the 6.3, 32, and 95 μg/L levels. The evaluation samples were therefore spiked with ethoxyquin at the 6.3 and 63 μg/L levels instead of at the 10 and 100 μg/L levels as originally assumed.

TABLE 4. ETHOXYQUIN CALIBRATION DATA

Concentration, μg/L	Average Peak Area	Standard Deviation, %	Number of Replicates	Average Response Factor
6.34	4.8	3	3	1.31
31.7	21.8	4	3	1.46
95.1	64.2	4	4	1.48

RESULTS AND DISCUSSION

Ethoxyquin was not detected in unspiked reagent water or POTW effluent samples. The chromatograms obtained from reagent water and POTW effluent blanks did not contain any other peaks resulting from interferences. This was presumably due to the fact that the fluorescence detection as opposed to UV detection was used. An example of a chromatogram obtained from HPLC analysis of an unspiked aliquot of POTW effluent is shown in Figure 3. Recoveries of ethoxyquin from reagent water were greater than 82 percent. Recoveries of ethoxyquin from POTW effluent were 58 percent or lower. Ethoxyquin recovery data are given in Table 5. Examples of chromatograms obtained from analyses of aliquots of POTW effluent unspiked and spiked with ethoxyquin at the 6.2 μg/L level are shown in Figure 3. Recoveries of ethoxyquin are generally lower from POTW effluent than from reagent water. It is likely that the ethoxyquin was adsorbing to particulate matter in the samples and was removed during the filtration step prior to HPLC analysis.

The EDL for ethoxyquin was determined by analyzing a 6.3 μg/L standard solution of ethoxyquin in HPLC mobile phase; the resultant chromatogram is shown in Figure 4. This solution yielded a S/N equal to 5.2. The EDL was calculated to be 6.1 μg/L of ethoxyquin in a sample, which is approximately six times the EDL of 1.0 μg/L reported in the method. The EDL does not take into account any interferences or recovery losses that might be encountered from a particular matrix.

RECOMMENDATIONS

The EDL determined for ethoxyquin was approximately six times higher than that reported in the ethoxyquin analysis method. This might be due to slight differences in instrumentation. However, literature references report a maximum excitation wavelength of 358 nm instead of 385 nm as specified in the method (14). Use of this wavelength might increase the sensitivity of the method.

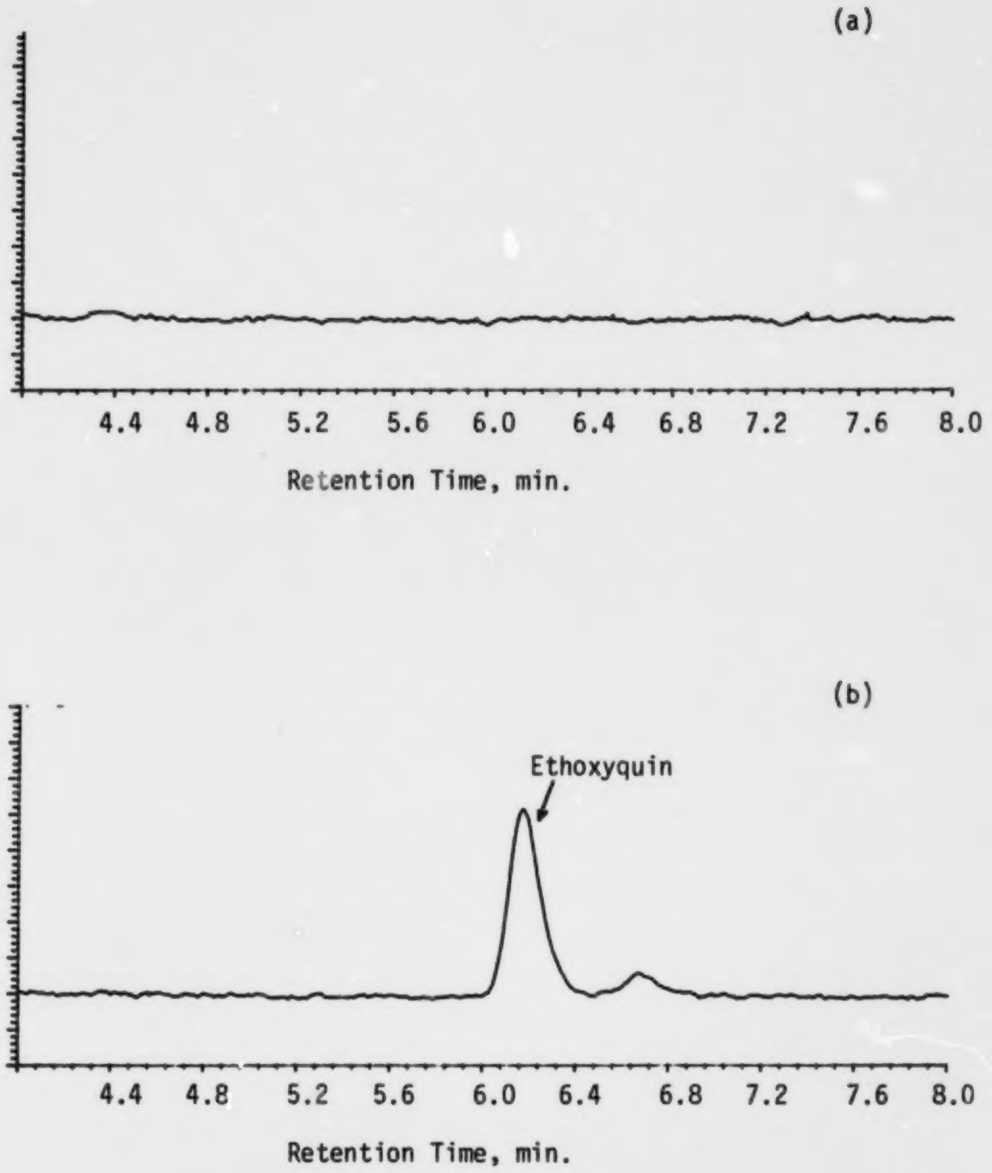


FIGURE 3. HPLC-FLUORESCENCE CHROMATOGRAM OF (a) POTW EFFLUENT, AND (b) POTW EFFLUENT SPIKED WITH ETHOXYQUIN AT THE 62 μg/L LEVEL.

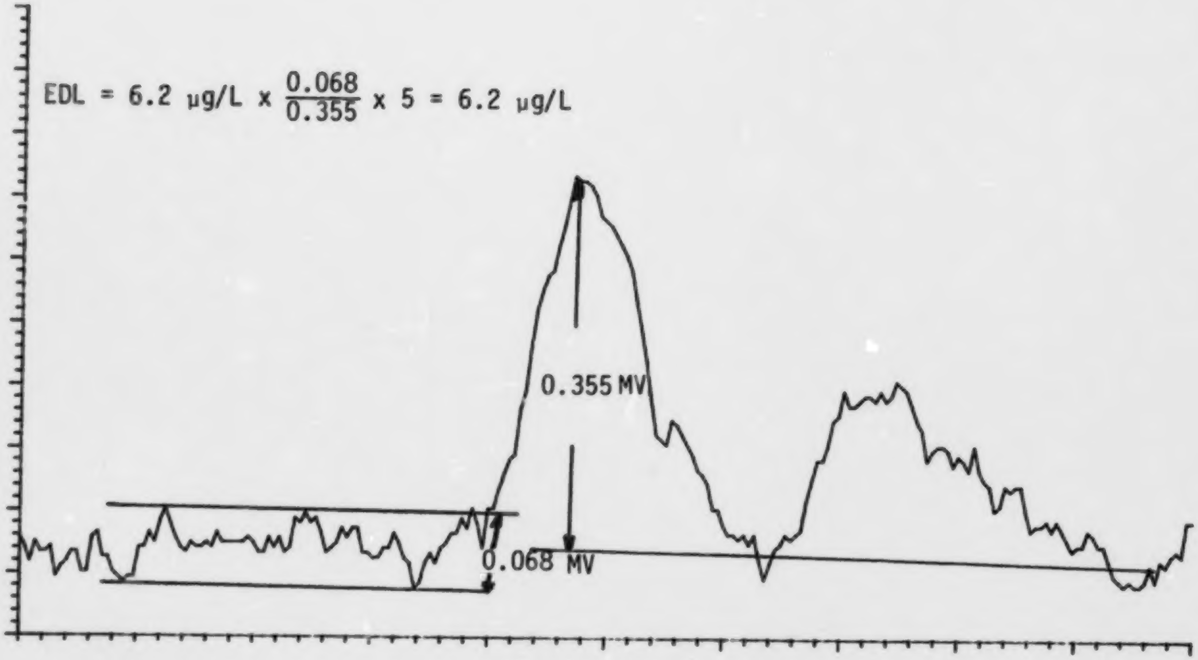

$$EDL = 6.2\ \mu g/L \times \frac{0.068}{0.355} \times 5 = 6.2\ \mu g/L$$

FIGURE 4. HPLC-FLUORESCENCE CHROMATOGRAM OF ETHOXYQUIN STANDARD EQUIVALENT TO 6.2 μg/L IN WATER USED TO DETERMINE METHOD EDL.

TABLE 5. RECOVERIES OF ETHOXYQUIN FROM WATER

Spike Level, µg/L	Matrix(a)	Blank Level, µg/L	Percent Recovery(c)	Standard Deviation, %
6.2	1	ND(b)	110	32(d)
62	1	ND	82	6
6.2	2	ND	19	14(d)
62	2	ND	58	1

(a) 1=Reagent water; 2=Columbus POTW secondary effluent.
(b) ND=not detected.
(c) Recovery data are averages of three replicate analyses.
(d) High standard deviation may be due to spike level of 6.2 µg/L which is close to the EDL of 6.3 µg/L.

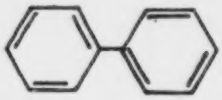
Studies in reagent water and POTW effluent revealed matrix dependent recovery losses. Although these recovery losses may be due to other characteristics of the POTW effluent, a probable cause is that the ethoxyquin becomes adsorbed to particulate matter in the POTW effluent and is removed during the filtration step of the method. Further studies should be conducted to ascertain if this is in fact the cause of the recovery losses. It might be possible to minimize adsorption of ethoxyquin onto particulate matter by adjusting matrix conditions so that the ethoxyquin is more likely to remain in the aqueous portion of the sample. Exactly how to do this is not obvious. Perhaps a more viable alternative is to remove the ethoxyquin from the aqueous sample by partitioning the sample with an organic solvent in which the ethoxyquin is preferentially soluble. This would also serve to remove particulate matter from the sample prior to HPLC analysis.

SECTION 3

ANALYSIS OF BIPHENYL AND O-PHENYLPHENOL IN WASTEWATER BY LIQUID CHROMATOGRAPHY

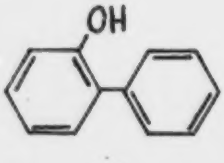
INTRODUCTION

Biphenyl (III) is used to inhibit mycelial growth and spore formation of citrus fruit roots. The CAS registry number for biphenyl is 92-52-4 and it is also referred to as 1,1'-biphenyl. Biphenyl has a melting point of 70.5°C, a boiling point of 256.1°C, and is practically insoluble in water. Biphenyl is stable in water under most temperature, pH, and light conditions. The acute oral LD50 for biphenyl for rats is 3280 mg/kg. Prolonged exposure to biphenyl vapors should be avoided (22).



III

O-phenylphenol (IV) is used as a disinfectant and fungicide. The CAS registry number for o-phenylphenol is 90-43-7, and it is commonly referred to as 2-phenylphenol, biphenyl-2-ol, 2-hydroxybiphenyl, (1,1'-biphenyl)-2-ol, and 2-biphenylol. O-phenylphenol forms colorless to pinkish crystals, has a melting point of 57 °C, a boiling point of 286 °C, and its water solubility is 0.7 g/kg at 25°C. O-phenylphenol is stable in water under most temperature, pH, and light conditions. The acute oral LD50 for o-phenylphenol for rats is 2480 mg/kg (23).



IV

An analysis method for determination of biphenyl and o-phenylphenol in produce has been reported (24). Produce samples are extracted by homogenizing them in acetone, and then by partitioning the homogenate with petroleum ether and methylene chloride. An adsorption chromatography cleanup procedure using Florisil was described; quantitative recoveries of biphenyl and o-phenylphenol were reported using this cleanup technique. Biphenyl and o-phenylphenol quantifications were done by packed column GC using a flame ionization detector (FID). A method to determine biphenyl in seawater has also been reported (25). Biphenyl was extracted from the seawater by equilibration with hexane or carbon tetrachloride in a separatory funnel. An adsorption chromatography cleanup procedure using silica gel or alumina was described. Analysis of biphenyl was done by capillary column GC-FID. Analysis methods using reverse-phase column HPLC-fluorescence, similar to the method evaluated, have also been reported (12,19).

The method provided by the Project Officer for evaluation for determination of biphenyl and o-phenylphenol in wastewaters consisted of extraction with methylene chloride in a separatory funnel; drying of the methylene chloride extracts over anhydrous sodium sulfate; solvent exchange to acetonitrile; concentration of the extract to 2.5 mL using Kuderna-Danish (K-D) techniques; and analysis of the extract by HPLC using reverse-phase conditions and a UV detector. The method included no cleanup procedure.

CONCLUSIONS

An analysis method designed to determine biphenyl and o-phenylphenol in wastewater was found to be marginally acceptable. The method was applied to reagent water and Columbus POTW effluent samples spiked at the 2.5 or 25 µg/L levels with biphenyl and o-phenylphenol. Recoveries of biphenyl and o-phenylphenol ranged from 51 to 82 percent; recoveries were not matrix-dependent. Losses may have been due to the rigorous conditions needed to concentrate the sample because of the use of acetonitrile as the solvent. The HPLC-UV conditions used for sample analyses yielded acceptable chromatography, sensitivity, and selectivity.

EXPERIMENTAL

The following procedure was outlined in the biphenyl and o-phenylphenol analysis method:

1. Place sample (approximately 1 liter) into a 2-liter separatory funnel and extract the sample with three 60-mL portions of methylene chloride. Combine the extracts.

2. The combined extracts are dried by pouring through a chromatographic column containing 10 cm of anhydrous sodium sulfate.

3. Concentrate the combined extracts to an apparent volume of 5 mL using K-D equipment with the water bath at 90 to 95°C.

4. Exchange the solvent to acetonitrile by adding 50 mL of acetonitrile to the extract in the K-D apparatus and concentrating as described above to 1 mL. Adjust the sample extract volume to 2.5 mL by addition of 1.5 mL of water.

5. Analyze the sample by HPLC-UV using the following conditions:
   - reverse-phase column, 2.6 mm ID by 250 mm long;
   - 10 micron Perkin-Elmer HC-ODS Sil-X;
   - isocratic elution for 5 minutes using 40 percent acetonitrile in water, then linear gradient elution to 100 percent acetonitrile over 25 minutes;
   - flow rate of 0.5 mL/min;
   - 50 μL injection volume;
   - UV detector at 254 nm.

Samples processed included the following:

1. Triplicate reagent water samples. Reagent water was obtained from a Millipore system.

2. Triplicate reagent water samples spiked with biphenyl and o-phenylphenol at the 2.5 μg/L concentration level.

3. Triplicate reagent water samples spiked with biphenyl and o-phenylphenol at the 25 μg/L concentration level.

4. Triplicate POTW secondary effluent samples. POTW secondary effluent was obtained from the City of Columbus.

5. Triplicate POTW secondary effluent samples spiked with biphenyl and o-phenylphenol at the 2.5 μg/L concentration level.

6. Triplicate POTW secondary effluent samples spiked with biphenyl and o-phenylphenol at the 25 μg/L concentration level.

The method EDLs, defined as the concentration of biphenyl and o-phenylphenol in a sample yielding a S/N of 5, were determined by injecting 0.01 and 0.05 μg/mL solutions of biphenyl and o-phenylphenol in HPLC mobile phase, respectively.

QUALITY ASSURANCE

Instrumentation was set up as described in the method. Sensitivity achieved was approximately equivalent to that reported in the method. Calibration standards were prepared containing biphenyl and o-phenylphenol at the 1.0, 10, and 50 μg/mL concentration levels in HPLC mobile phase. These are equivalent to biphenyl and o-phenylphenol concentrations of 2.5, 25, and 125 μg/L in a water sample. These calibration standards were analyzed in duplicate prior to analyses of the water extracts; a calibration standard was analyzed after every five water extracts. Response factors were calculated for each calibration run by dividing the concentration level in μg/L by the corresponding peak area. Resultant calibration data are reported in Table 6. Response factors were repeatable over the entire calibration range. Response factors of 0.00073 and 0.00016 were used for calculations of biphenyl and o-phenylphenol concentrations in the water extracts, respectively.

TABLE 6. BIPHENYL AND O-PHENYLPHENOL CALIBRATION DATA

Compound	Concentration μg/L[(a)]	Average Peak Area	Percent Standard Deviation	Number of Replicates	Average Response Factors
Biphenyl	2.5	3520	1	3	0.00071
Biphenyl	25	33700	5	4	0.00074
Biphenyl	125	166000	1	4	0.00075
O-phenylphenol	2.5	1600	8	3	0.0016
O-phenylphenol	25	15800	1	4	0.0016
O-phenylphenol	125	79100	2	4	0.0016

(a) Equivalent concentration of compound in water sample.

Standards of biphenyl and o-phenylphenol were obtained from two independent sources. Calibration standards used for quantification of biphenyl and o-phenylphenol in the water samples were prepared from compounds obtained from the reference standards repository of EPA-HERL. A test calibration standard was prepared at the 10 μg/mL concentration level using biphenyl and o-phenylphenol from a chemical supply house. This test calibration standard was analyzed in triplicate and the resultant peak areas were compared to those generated from analyses of the original calibration standards. For both compounds, the average peak area generated from the test calibration standard varied by less than six percent from the peak areas generated from the original calibration solutions.

RESULTS AND DISCUSSION

Recoveries of biphenyl and o-phenylphenol from reagent water and POTW effluent were generally less than 80 percent. These recovery data are given in Table 7. The cause of these low recoveries is not evident. The extraction procedure used should provide efficient removal of these two compounds from the water samples. The conditions required to concentrate the samples to

TABLE 7. RECOVERIES OF BIPHENYL AND O-PHENYLPHENOL FROM WATER[(a)]

Compound	Spike Level, µg/L	Matrix[(b)]	Blank Level, µg/L	Percent Recovery[(c)]	Standard Deviation, %
Biphenyl	2.5	1	0.20	74	2
Biphenyl	25	1	0.20	51	11
Biphenyl	2.5	2	0.90	56	4
Biphenyl	25	2	0.90	61	1
O-phenylphenol	5.0	1	ND[(d)]	73	4
O-phenylphenol	50	1	ND	60	5
O-phenylphenol	5.0	2	ND	69	2
O-phenylphenol	50	2	ND	82	15

(a) Recovery data are averages of three replicate analyses.
(b) 1=Reagent water; 2=Columbus POTW secondary effluent.
(c) Corrected for blank level.
(d) ND=not detected.

2.5 mL after addition of acetonitrile are rigorous. The high bath temperatures necessary for K-D concentrations may contribute to the loss of these compounds. A pH adjustment step was not included in the method. Reagent water and POTW effluent samples used were generally in a pH range of 5-6. A lower pH might increase recoveries of o-phenylphenol by increasing its extraction efficiency from water.

Both reagent water and POTW effluent samples contained low levels, 0.2 to 0.9 µg/L, of biphenyl or a compound eluting at the same time as biphenyl. These interferences did not complicate the determination of biphenyl in the samples. O-phenylphenol was not detected in any of the unspiked samples. Examples of chromatograms obtained from reagent water and POTW secondary effluent samples unspiked and spiked at the 2.5 µg/L level are given in Figures 5 and 6.

The method EDLs were determined by analyzing 0.01 and 0.05 µg/mL solutions of biphenyl and o-phenylphenol in HPLC mobile, respectively; the resultant chromatograms are shown in Figures 7 and 8. These solutions yielded S/Ns of 14 and 9.3, respectively. The EDLs for biphenyl and o-phenylphenol were determined to be 0.04 and 0.01 µg/L, respectively. These EDLs were significantly lower than the EDLs of 0.25 and 0.5 µg/L reported in the method for biphenyl and o-phenylphenol, respectively.

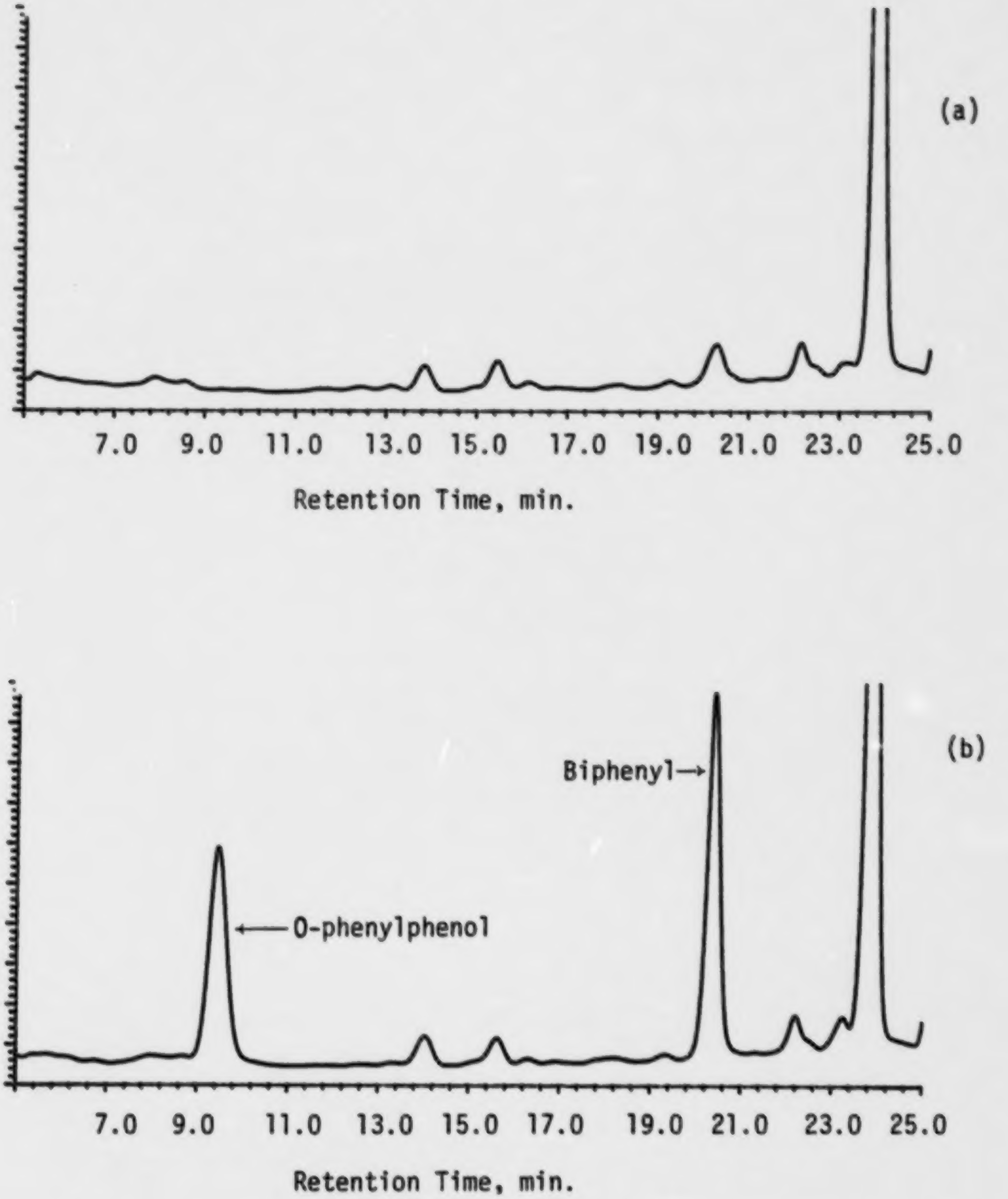


FIGURE 5. HPLC-UV CHROMATOGRAMS OF (a) REAGENT WATER AND (b) REAGENT WATER SPIKED WITH BIPHENYL AND O-PHENYLPHENOL AT THE 2.5 µg/L LEVEL.

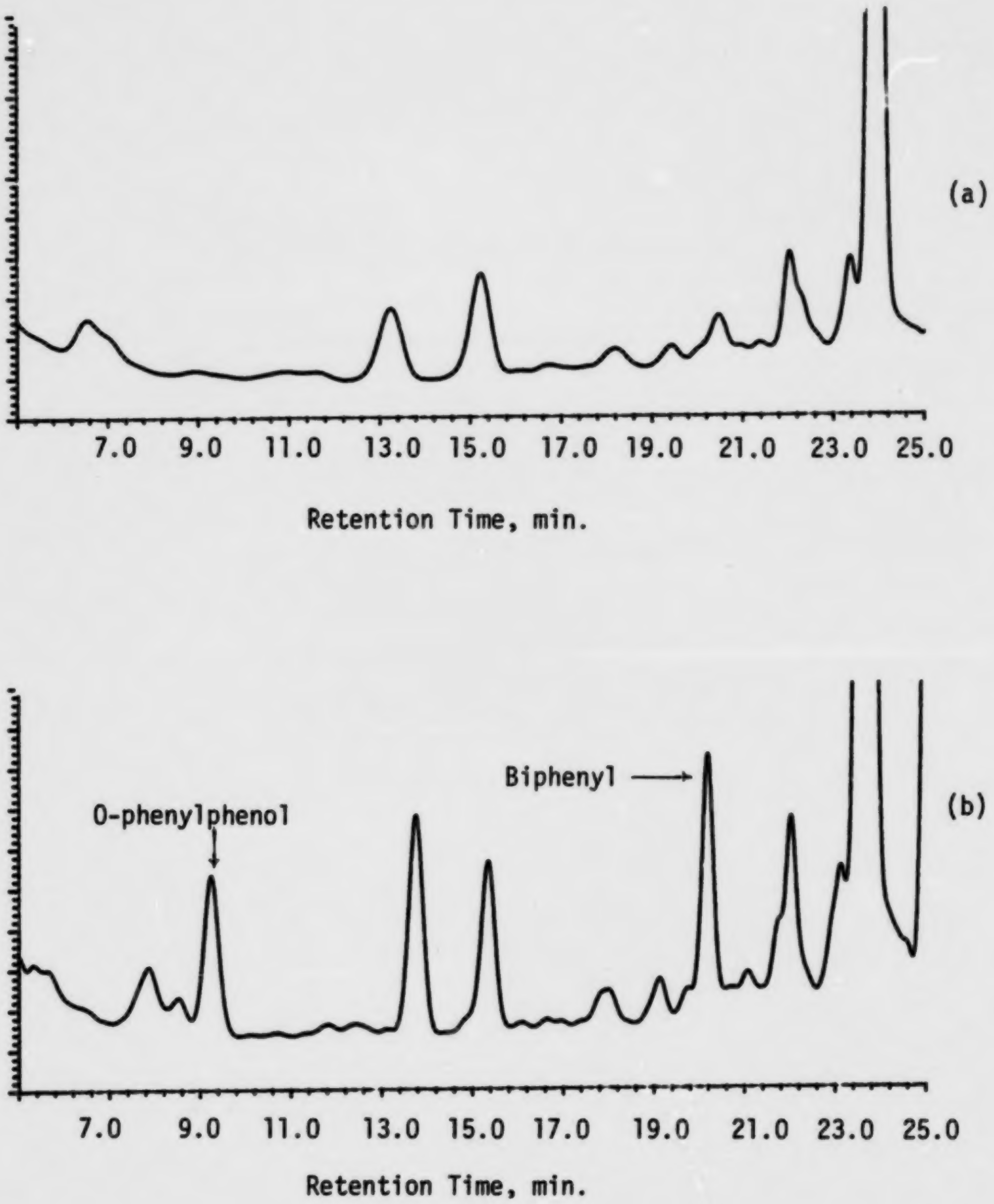
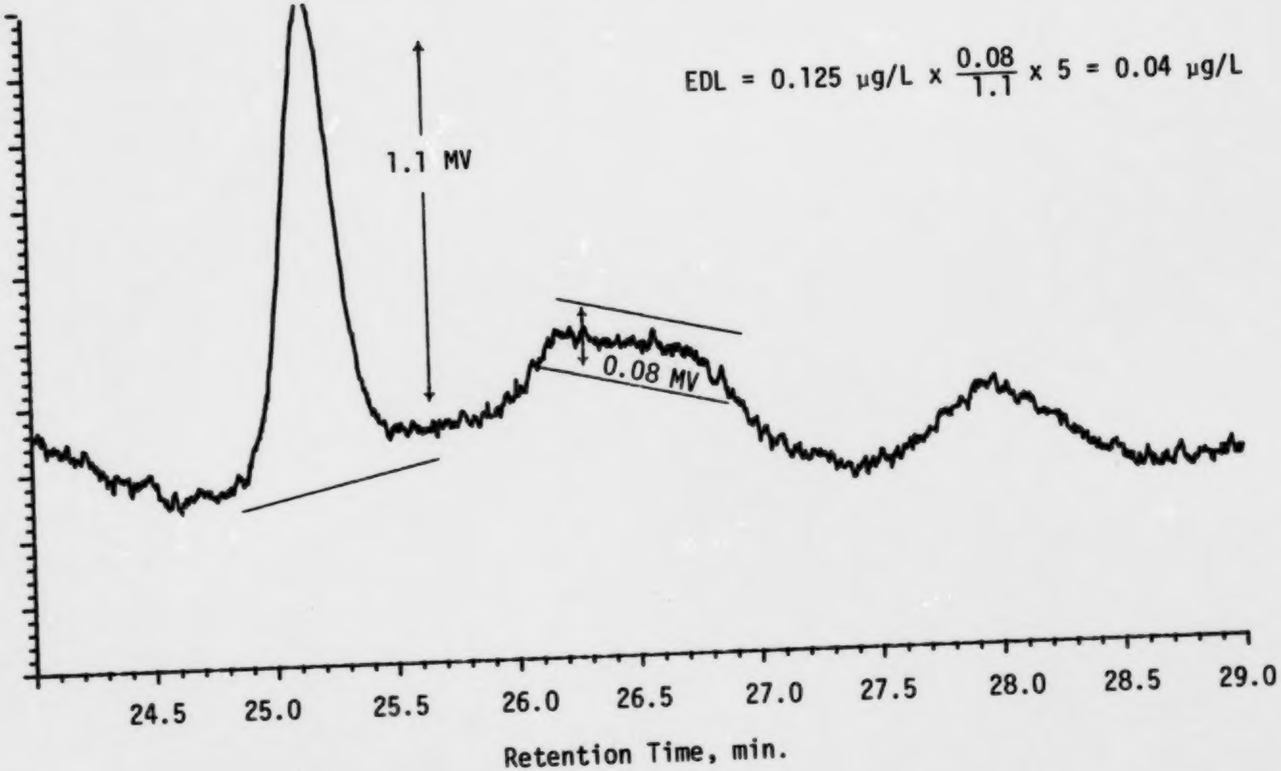


FIGURE 6. HPLC-UV CHROMATOGRAMS OF (a)POTW EFFLUENT AND (b) POTW EFFLUENT SPIKED WITH BIPHENYL AND O-PHENYLPHENOL AT THE 2.5 μg/L LEVEL.



$$EDL = 0.125\ \mu g/L \times \frac{0.08}{1.1} \times 5 = 0.04\ \mu g/L$$

FIGURE 7. HPLC-UV CHROMATOGRAM OF A BIPHENYL STANDARD EQUIVALENT TO 0.125 μg/L IN WATER USED TO DETERMINE METHOD EDL.

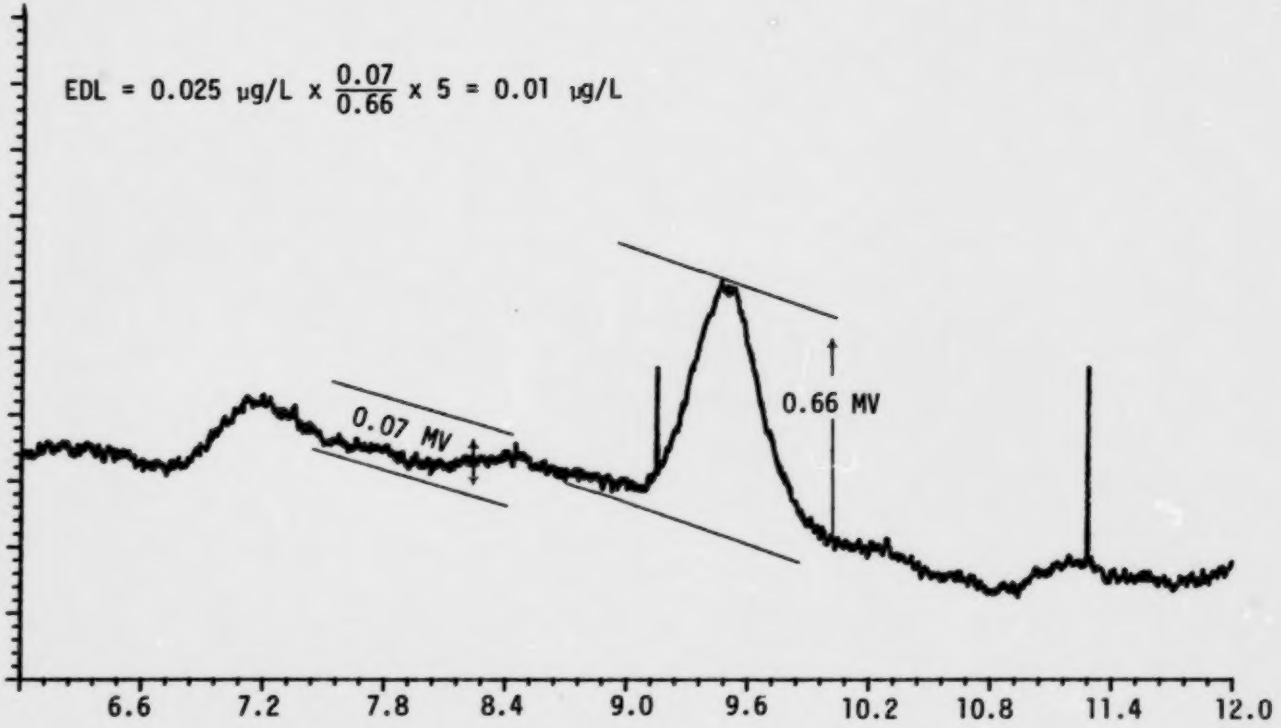


FIGURE 8. HPLC-UV CHROMATOGRAM OF AN O-PHENYLPHENOL STANDARD EQUIVALENT TO 0.025 μg/L IN WATER USED TO DETERMINE METHOD EDL.

RECOMMENDATIONS

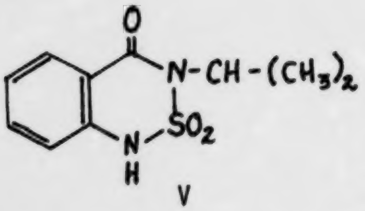
It is possible that the low recoveries of biphenyl and o-phenylphenol may have resulted from problems encountered during the extraction and/or concentration steps of the analysis method. Further study is warranted to improve the recoveries of these two compounds from water. Studies should be directed towards optimizing the extraction conditions, perhaps by adjusting the sample pH or by using extraction procedures that are more easily controlled than the use of separatory funnels. A suggested alternative is to tumble the water/methylene chloride mixture for a prescribed amount of time. The concentration conditions should also be changed so that they are not so rigorous. This might be done by solvent exchange to a lower-boiling solvent than acetonitrile, or using a concentration apparatus other than K-D equipment.

SECTION 4

ANALYSIS OF BENTAZON IN WASTEWATER BY LIQUID CHROMATOGRAPHY

INTRODUCTION

Bentazon (V) is used as a contact herbicide. The IUPAC name for bentazon is 3-isopropyl-(1H)-benzo-2,1,3-thiadiazin-4-one-2,2-dioxide, and its CAS registry number is 25057-89-0. Common synonyms for bentazon include BAS 351H, Basagran, Bentazone, and Bendioxide. Bentazon is a colorless crystalline powder with a melting point of 137-139°C. Bentazon has a solubility in water of 500 mg/kg at 20°C. The acute oral LD50 for rats for bentazon is 1100 mg/kg (26).



V

Methods have been reported for the determination of bentazon in soil (27,28). The soil sample was shaken with a mixture of acetonitrile, water, and methanol; the extracted bentazon was derivatized with diazomethane and analyzed by GC-ECD. Other GC methods have been reported (29). A reverse-phase HPLC-UV analysis method similar to the method validated has also been reported (30). Literature references indicate that bentazon may decompose when exposed to light (29). No cleanup methods were reported.

The method provided by the Project Officer for evaluation for determination of bentazon in wastewaters consisted of adjustment of the sample pH by the addition of sodium hydroxide; addition of sodium chloride to the sample; extraction of the sample with methylene chloride using a separatory funnel; back extraction of the methylene chloride extract with 0.1 M NaOH; and adjustment of the sample volume to 5 mL with acetate buffer.

CONCLUSIONS

An analysis method designed to determine bentazon in wastewaters was found to be acceptable. The method was applied to reagent water and Columbus POTW effluent samples spiked at the 10 and 100 µg/L levels with bentazon. Recoveries of bentazon from all of the samples were greater than 76 percent. The HPLC-UV conditions used for sample analyses yielded acceptable chromatography, sensitivity, and selectivity.

EXPERIMENTAL

The following procedure was outlined in the bentazon analysis method:

1. Place sample (approximately 1 liter) into a 2-liter separatory funnel; add 35 mg/L of sodium thiosulfate per ppm of free chlorine; adjust the pH of the sample to within the range of 2.5 to 3.5 with dilute sodium hydroxide or sulfuric acid; and add 200 grams of sodium chloride.

2. Extract the sample with three 60-mL portions of methylene chloride, combining the extracts in a 250-mL separatory funnel.

3. Extract the methylene chloride extract with two 2-mL aliquots of 0.1 M NaOH in HPLC water and transfer the aqueous layers to a 5-mL volumetric flask.

4. Add two drops of glacial acetic acid to the volumetric flask and dilute to volume with acetate buffer solution.

5. Analyze the sample by HPLC with UV detection using the following conditions:
   - reverse-phase Ultrasphere ODS, 4.6 mm ID by 250 mm column;
   - isocratic 35 percent methanol/65 percent buffer (pH 4.7) mobile phase;
   - flow rate of 2.0 mL/min;
   - injection volume of 100 µL;
   - UV detector wavelength set at 340 nm.

No modifications were made in this method. The HPLC column particle size was not specified in the method. In consultation with the EPA Task Officer, prior to method evaluation, it was specified that a 5 micron Ultrasphere ODS column should be used.

Samples processed included the following:

1. Triplicate reagent water samples. Reagent water was obtained from a Millipore system.

2. Triplicate reagent water samples spiked with bentazon at the 10 µg/L concentration level.

3. Triplicate reagent water samples spiked with bentazon at the 100 µg/L concentration level.

4. Triplicate POTW secondary effluent samples. POTW secondary effluent was obtained from the City of Columbus.

5. Triplicate POTW secondary effluent samples spiked with bentazon at the 10 µg/L concentration level.

6. Triplicate POTW secondary effluent samples spiked with bentazon at the 100 µg/L concentration level.

Recoveries were determined by comparison to standard solutions of bentazon prepared at the 1, 5, and 50 µg/mL concentration levels in HPLC mobile phase. These concentration levels were equivalent to 5, 25, and 250 µg/L of bentazon in the original samples assuming a final extract volume of 5 mL. Response factors were calculated for the standards and used to determine concentration levels in the abovementioned reagent water and POTW effluent samples.

The EDL for bentazon, defined as the concentration of bentazon in a sample yielding a S/N of 5, was determined by injecting a 0.1 µg/mL standard solution of bentazon prepared in HPLC mobile phase. The solution was equivalent to a bentazon concentration of 0.5 µg/L in a water sample.

QUALITY ASSURANCE

Instrumentation was set up as described in the method. Sensitivity achieved was approximately equivalent to that reported in the method. Calibration standards consisted of bentazon solutions prepared at the 1, 5, and 25 µg/mL concentration levels in HPLC mobile phase. Each calibration standard was analyzed in duplicate prior to analysis of any evaluation samples. A selected calibration standard was then analyzed after every five evaluation samples. Response factors were calculated for each calibration run by dividing the equivalent bentazon concentration level in the sample in µg/L by the bentazon peak area. Resultant calibration data are reported in Table 8. Response factors were reproducible over the entire calibration range. A response factor of 0.10 was used for calculations of bentazon concentrations in the evaluation samples.

Standards of bentazon were obtained from two independent sources. Bentazon from the reference standards repository of the EPA-HERL was used for preparation of calibration standards as well as for preparation of the evaluation samples. A test calibration standard was also prepared at the 5 µg/L concentration level from bentazon from a chemical supply house. The peak area resulting from the analysis of the test calibration standard was less than two percent lower than the average peak area obtained from analyses of the equivalent EPA-HERL calibration standard indicating that the standards obtained were probably within two percent of their specified purities.

TABLE 8. BENTAZON CALIBRATION DATA

Concentration, μg/L[(a)]	Average Peak Area	Standard Deviation	Number of Replicates	Average Response Factors
5.0	52.4	1.34	3	0.10
25	224	1.66	3	0.11
250	2490	2.23	4	0.10

(a) Equivalent concentration of bentazon in water sample.

RESULTS AND DISCUSSION

Bentazon was not detected in unspiked reagent water or POTW effluent samples. The chromatograms obtained from reagent water and POTW effluent blanks did not contain any other peaks resulting from interferences. This was unexpected since the use of UV detectors usually results in some evidence of interferences from the sample matrix. Apparently the basic backextraction step specified in the extraction procedure removes any potential interferences in the matrices used for this evaluation. Recoveries of bentazon from both reagent water and POTW effluent were greater than 92 percent at the 10 μg/L spike level. Recoveries were slightly lower from both samples at the 100 μg/L level, ranging from 76 to 81 percent. Since spiked water samples were prepared using the same stock standards, it is not expected that the recovery differences between the two spike levels are due to errors during sample preparation. Bentazon recovery data are given in Table 9. Examples of chromatograms obtained from the analyses of aliquots of POTW effluent unspiked and spiked with bentazon at the 10 μg/L level are shown in Figure 9.

The EDL for bentazon was determined by analyzing a 0.1 μg/mL standard solution of bentazon in HPLC mobile phase; the resultant chromatogram is shown in Figure 10. This solution yielded a S/N of 2.3. The EDL was calculated to be 1.1 μg/L of bentazon, which is the same value reported in the method. The EDL does not take into account any interferences or recovery losses that might be encountered from a particular matrix.

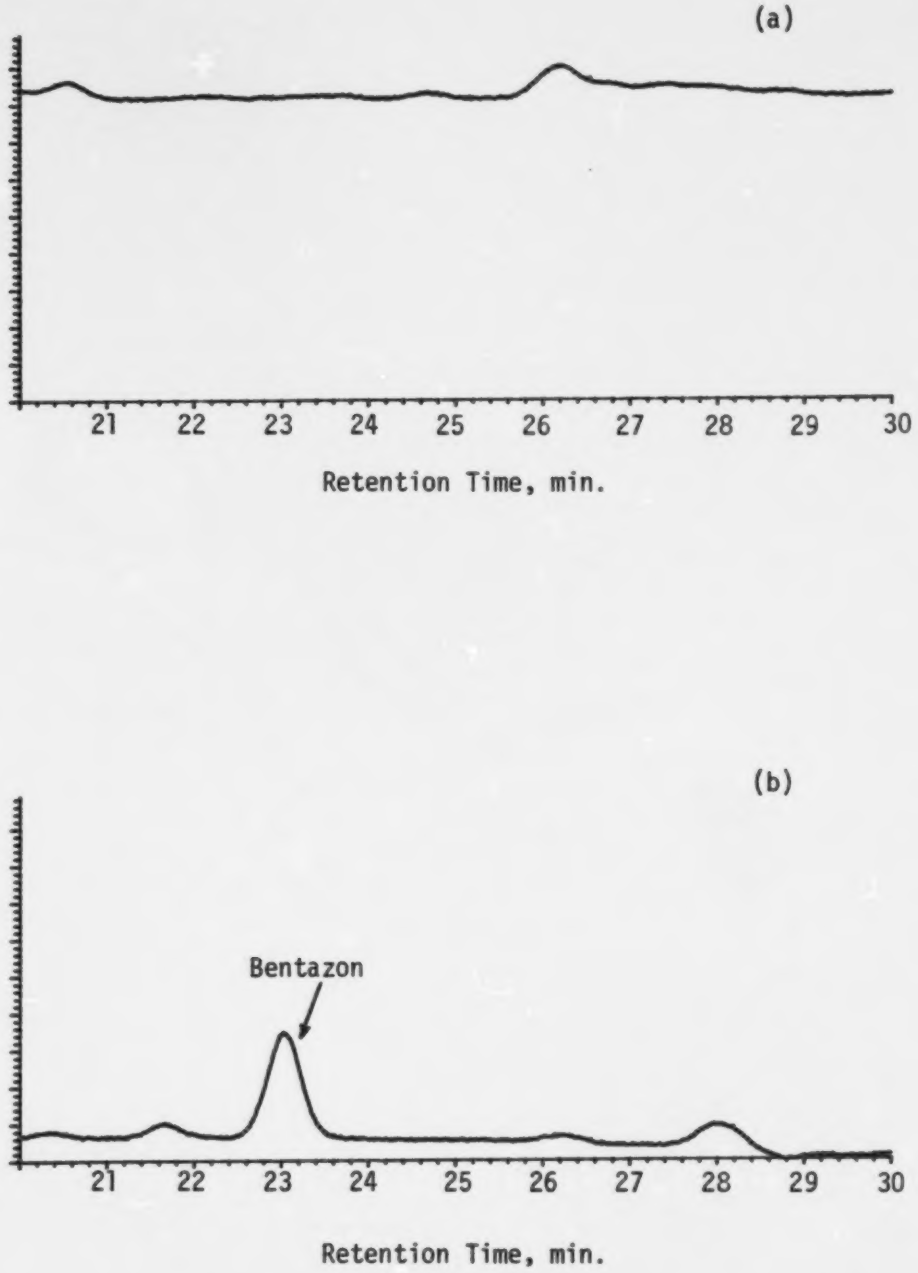


FIGURE 9. HPLC-UV CHROMATOGRAM OF (a) UNSPIKED POTW EFFLUENT, AND (b) POTW EFFLUENT SPIKED WITH BENTAZON AT THE 10 µg/L LEVEL

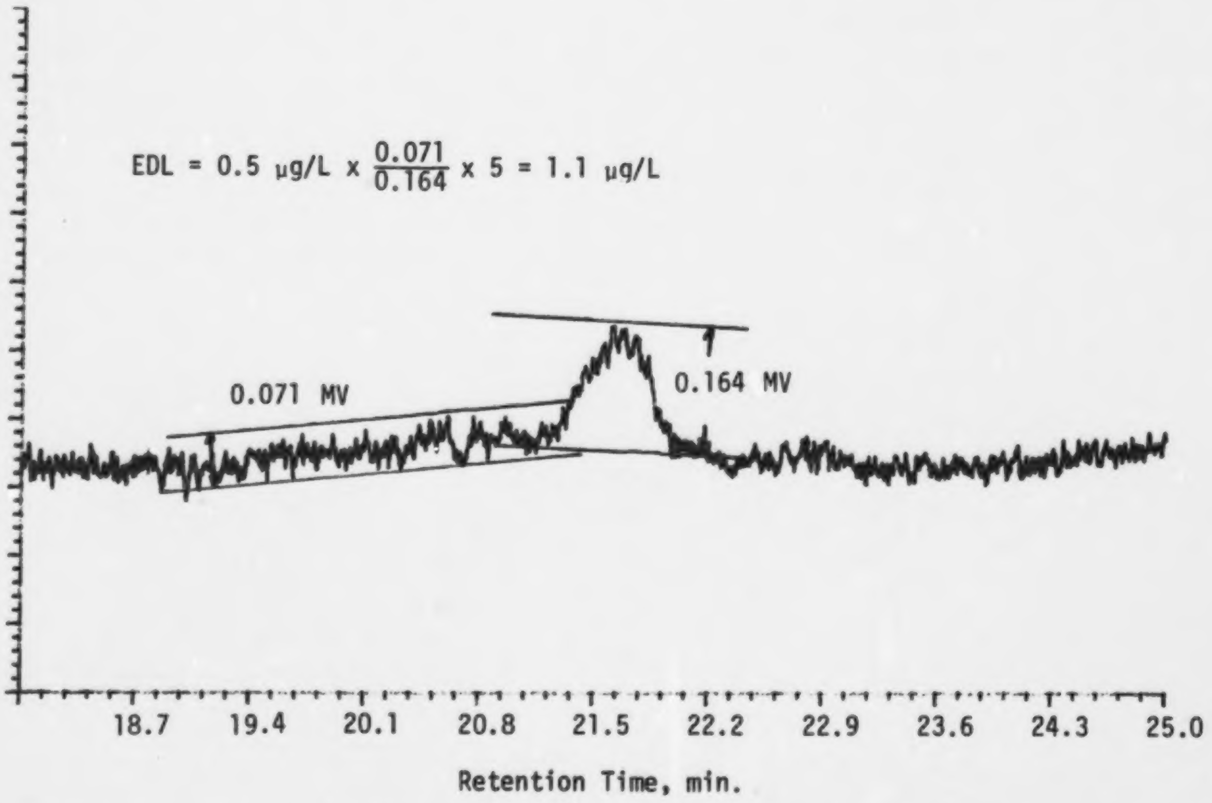


FIGURE 10. HPLC-UV CHROMATOGRAM OF A BENTAZON STANDARD EQUIVALENT TO 0.5 µg/L IN WATER USED FOR EDL DETERMINATION

TABLE 9. RECOVERIES OF BENTAZON FROM WATER

Spike Level, µg/L	Matrix[(a)]	Blank Level, µg/L	Percent Recovery[(c)]	Standard Deviation, %
10	1	ND[(b)]	92	2
100	1	ND	81	10
10	2	ND	94	7
100	2	ND	76	2

(a) 1=Reagent water; 2=Columbus POTW secondary effluent.
(b) ND=not detected.
(c) Recovery data are averages of three replicate analyses.

RECOMMENDATIONS

The method was acceptable for determination of bentazon in reagent water and POTW effluents. Lower recoveries were observed at the 100 µg/L bentazon spike levels than were observed at the 10 µg/L bentazon spike levels. Although this might have been caused by spiking irregularities, this is not thought to be the case. The solubility of bentazon in water at 20°C is approximately 500 µg/mL. The concentration of bentazon in the final extract derived from a one-liter sample of water spiked at the 20 µg/L level is 100 µg/mL. This concentration level is not close to the solubility limits of bentazon in water; recovery losses were most likely not due to precipitation of bentazon from the sample.

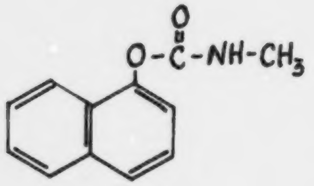
SECTION 5

ANALYSIS OF CARBAMATE AND AMIDE PESTICIDES IN WASTEWATER BY LIQUID CHROMATOGRAPHY

INTRODUCTION

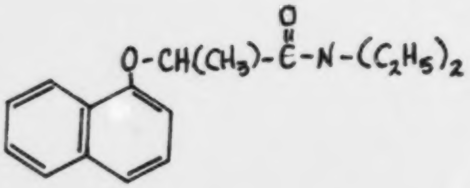
Pesticides included in the method evaluated were carbaryl, napropamide, propanil and vacor. A standard of vacor could not be obtained; the method was not evaluated for vacor.

Carbaryl (VI) is a contact insecticide with slight systemic properties. The IUPAC name for carbaryl is 1-naphthyl methylcarbamate and its CAS registry number is 63-25-2. A common synonym for carbaryl is Sevin. Carbaryl is a colorless crystalline solid with a melting point of 142°C. Carbaryl has a solubility in water of 120 mg/L at 30°C; it is soluble in most polar organic solvents. Carbaryl is stable to light, heat and hydrolysis under normal storage conditions. The acute oral LD50 for male rats for carbaryl is 850 mg/kg (31).



VI

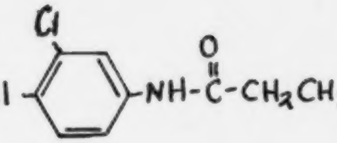
Napropamide (VII) is an herbicide. The IUPAC name for napropamide is N,N-diethyl-2-(1-naphthyloxy)propionamide and its CAS registry number is 15299-99-7. A common synonym for napropamide is Devrinol. Napropamide is



VII

a brown solid with a melting point of 74.8-75.5°C. Napropamide has a solubility in water of 73 mg/L at 20°C and is very soluble in acetone, ethanol, and xylene. The acute oral LD50 for rats for napropamide is >5000 mg/kg (32).

Propanil (VIII) is a contact herbicide. The IUPAC name for propanil is 3',4'-dichloropropionanilide and its CAS registry number is 709-98-8. Common synonyms for propanil include Stam F-34, Surcopur, and Rogue. Propanil is a colorless solid with a melting point of 92-93°C. Propanil has a solubility in water of 225 mg/L at 25°C. Propanil is hydrolyzed in acid and alkaline media to 3,4-dichloroaniline and propionic acid. The acute oral LD50 for rats for propanil is 1285-1483 mg/kg (96).



VIII

Analysis methods for the determination of carbaryl, napropamide, and propanil in produce samples have been reported in the literature. These methods usually involve the use of reverse-phase column HPLC coupled with a UV or fluorescence detector (19,30,33,34,35). Several analysis methods using packed or capillary column GC, usually with an electron capture detector, are also reported (24,36,37,38,39).

The method provided by the Project Officer for evaluation for determination of carbamate and amide pesticides consisted of addition of NaCl to the sample; adjustment of the sample pH by addition of dilute sodium hydroxide or sulfuric acid; extraction of the sample with methylene chloride using a separatory funnel; drying of the sample using anhydrous sodium sulfate; concentration of the sample to 10 mL after solvent substitution with HPLC mobile phase; and analysis of the sample extract by reverse phase HPLC using a UV detector.

CONCLUSIONS

A method designed to determine carbamate and amide pesticides in wastewaters was evaluated. The method was found to be acceptable when applied to two of the compounds, napropamide and propanil. The method was not acceptable for the determination of carbaryl in wastewater. The instrumentation yielded sufficient sensitivity to determine low µg/L levels of all three compounds in a water matrix. Recoveries of napropamide and propanil from water were generally greater than 80 percent. However, recoveries of carbaryl were generally lower than 50 percent and were matrix and concentration dependent.

EXPERIMENTAL

The following procedure was outlined in the carbamate and amide analysis method:

1. Place sample (approximately 1 liter) into a 2-liter separatory funnel; add 200 g sodium chloride to the sample; and adjust the pH of the sample to within the range of 6.5 to 7.5 with dilute sodium hydroxide or sulfuric acid.
2. Extract the sample with three 60-mL portions of methylene chloride, combining the extracts.
3. Dry the combined extracts by pouring through a chromatographic column containing 10 cm of anhydrous sodium sulfate.
4. Concentrate the combined extracts to an apparent volume of 1 mL using a rotating evaporator with the water bath temperature between 35 and 40°C.
5. Add 15 mL of acetonitrile to the sample and reconcentrate to an apparent volume of 1 mL using the rotating evaporator.
6. Adjust sample volume to 10 mL with 50:50 acetonitrile:water.
7. Analyze the sample by HPLC-UV using the following conditions:
   - reverse-phase column, 4 mm ID by 250 mm long;
   - 5 micron Ultrasphere ODS;
   - linear gradient elution from 40 percent acetonitrile in water to 65 percent acetonitrile in water over 10 minutes;
   - flow rate of 1 mL/min;
   - 100 µL injection volume;
   - UV detector at 254 nm.

Samples processed included the following:

1. Triplicate reagent water samples. Reagent water was obtained from a Millipore system.
2. Triplicate reagent water samples spiked with carbaryl, napropamide, and propanil at the 2.0, 6.0, and 0.2 µg/L concentration levels, respectively.
3. Triplicate reagent water samples spiked with carbaryl, napropamide, and propanil at the 20, 60, and 2.0 µg/L concentration levels, respectively.
4. Triplicate POTW secondary effluent samples. POTW secondary effluent was obtained from the City of Columbus.

5. Triplicate POTW secondary effluent samples spiked with carbaryl, napropamide, and propanil at the 2.0, 6.0, and 0.2 µg/L concentration levels, respectively.

6. Triplicate POTW secondary effluent samples spiked with carbaryl, napropamide, and propanil at the 20, 60, and 2.0 µg/L concentration levels, respectively.

Recoveries were determined by comparison to standard solutions of carbaryl and napropamide prepared at the 0.1, 1.0, and 10 µg/mL concentration levels and propanil at the 0.01, 0.05, and 0.2 µg/mL concentration levels in HPLC mobile phase. These concentration levels were equivalent to 1.0, 10, and 100 µg/L of carbaryl and napropamide, and 0.1, 0.5, and 2.0 µg/L of propanil in the original samples assuming a final extract volume of 10 mL. Response factors were calculated for the standards and used to determine concentration levels in the abovementioned reagent water and POTW effluent samples.

The method EDLs, defined as the concentrations of carbamate and amide pesticides in a sample yielding a S/N of 5, were determined by injecting 0.05, 0.005, and 0.5 µg/L solutions of carbaryl, napropamide, and propanil in HPLC mobile phase, respectively.

QUALITY ASSURANCE

Instrumentation was set up as described in the method. Sensitivity achieved was approximately equivalent to that reported in the method. Calibration standards were prepared containing carbaryl and napropamide at the 0.1, 1.0, and 10 µg/mL concentration levels and propanil at the 0.01, 0.05, and 0.2 µg/mL concentration levels in HPLC mobile phase. These are equivalent to carbaryl and napropamide concentrations of 1.0, 10, and 100 µg/L and propanil concentrations of 0.1, 0.5, and 2.0 µg/L in a water sample. These calibration standards were analyzed in duplicate prior to analyses of the water extracts; a calibration standard was analyzed after every five water extracts. Response factors were calculated for each calibration run by dividing the concentration level in µg/L by the corresponding peak area. Resultant calibration data are reported in Table 10. Response factors were repeatable over the entire calibration range. Response factors of 0.10, 0.17, and 0.018 were used for calculations of carbaryl, napropamide, and propanil concentrations in the water extracts, respectively.

Standards of carbaryl, napropamide, and propanil were obtained from two independent sources. Calibration standards used for quantification of these compounds in the water samples were prepared from compounds obtained from the reference standards repository of EPA-HERL. A test standard was prepared at the 10 µg/mL concentration level for carbaryl and napropamide and at the 0.2 µg/mL concentration level for propanil in the HPLC mobile phase using compounds obtained from a chemical supply house. This test calibration standard was analyzed in duplicate and the resultant peak areas compared to those generated from analyses of the original calibration standards. Resultant data indicated that in all cases the standard obtained from the chemical supply

TABLE 10. CARBARYL, NAPROPAMIDE, AND PROPANIL CALIBRATION DATA

Compound	Concentration, µg/L[(a)]	Average Peak Area	Standard Deviation %	Number of Replicates	Average Response Factors
Carbaryl	1.0	5.3	23	2	0.19
Carbaryl	10	50.9	4	4	0.20
Carbaryl	100	517	1	4	0.19
Napropamide	1.0	6.1	13	2	0.16
Napropamide	10	57.0	8	3	0.18
Napropamide	100	570	1	4	0.18
Propanil	0.1	5.5	6	2	0.018
Propanil	0.5	32.8	8	4	0.015
Propanil	2.0	102	2	4	0.020

(a) Equivalent concentration of compound in water sample.

house was not as pure as that obtained from the reference standards repository. Peak areas obtained from the test calibration standards of carbaryl, napropamide, and propanil were 36 percent, 18 percent, and 12 percent lower than those obtained from the original calibration standards, respectively.

RESULTS AND DISCUSSION

Recoveries of carbaryl from reagent water and POTW effluent were low. Recoveries of carbaryl were lower from water spiked at the 2 µg/L level than from water spiked at the 20 µg/L level. Recoveries of carbaryl from POTW effluent were lower than that from reagent water spiked at the same concentration levels. Recoveries of napropamide and propanil were generally greater than 80 percent from both reagent water and POTW effluent, regardless of the compound concentration level. These recovery data are given in Table 11. Examples of chromatograms obtained from the HPLC analyses of unspiked and spiked reagent water and POTW effluent samples are shown in Figures 11 and 12. The cause of the low carbaryl recoveries is not evident. Two sources of recovery losses can be examined: carbaryl may not be efficiently extracted from the water into the methylene chloride during the extraction procedure and/or carbaryl may be lost during the concentration step.

POTW effluent samples contained low levels of napropamide and propanil or compounds eluting at the same times. These napropamide and propanil interferences were present at the 1.7 and 0.06 µg/L levels, respectively. These interferences did not complicate the determination of napropamide or propanil in the samples. Carbaryl was not detected in the unspiked POTW effluent. No interferences were detected in the reagent water.

TABLE 11. RECOVERIES OF CARBARYL, NAPROPAMIDE, AND PROPANIL FROM WATER(c)

Compound	Spike Level, µg/L	Matrix(a)	Blank Level, µg/L	Percent Recovery(d)	Standard Deviation ,%
Carbaryl	2.0	1	ND(b)	52	10
Carbaryl	20	1	ND	83	13
Carbaryl	2.0	2	ND	ND	--
Carbaryl	20	2	ND	28	16
Napropamide	6.0	1	ND	103	2
Napropamide	60	1	ND	102	2
Napropamide	6.0	2	1.7	96	7
Napropamide	60	2	1.7	94	3
Propanil	0.2	1	ND	79	6
Propanil	2.0	1	ND	99	3
Propanil	0.2	2	0.06	85	11
Propanil	2.0	2	0.06	77	7

(a) 1=Reagent water; 2=Columbus POTW secondary effluent.
(b) ND = not detected.
(c) Recovery data are averages of three replicate analyses.

The HPLC-UV system was approximately ten times less sensitive for carbaryl than stated in the method. System sensitivity for propanil and napropamide was approximately equal to that listed in the method. The method EDLs were determined by injecting 0.05, 0.005, and 0.05 µg/mL solutions of carbaryl, napropamide, and propanil in HPLC mobile phase, respectively; resultant chromatograms are shown in Figures 13-15. These carbaryl, napropamide, and propanil solutions yielded S/Ns of 2.1, 13, and 8.3, respectively. The EDLs for carbaryl, napropamide, and propanil were determined to be 1.2, 0.02, and 0.3 µg/L, respectively.

RECOMMENDATIONS

The analysis method was acceptable for the determination of napropamide and propanil in reagent water and POTW effluents. Recoveries of carbaryl, however, were low enough to warrant further examination of the method as applied to carbaryl; possibly carbaryl should be removed from the method. Carbaryl is more polar than napropamide and propanil and is most likely not as efficiently removed from water into methylene chloride. The use of a more polar extraction solvent, or perhaps more exhaustive extraction of the water sample might increase carbaryl recoveries.

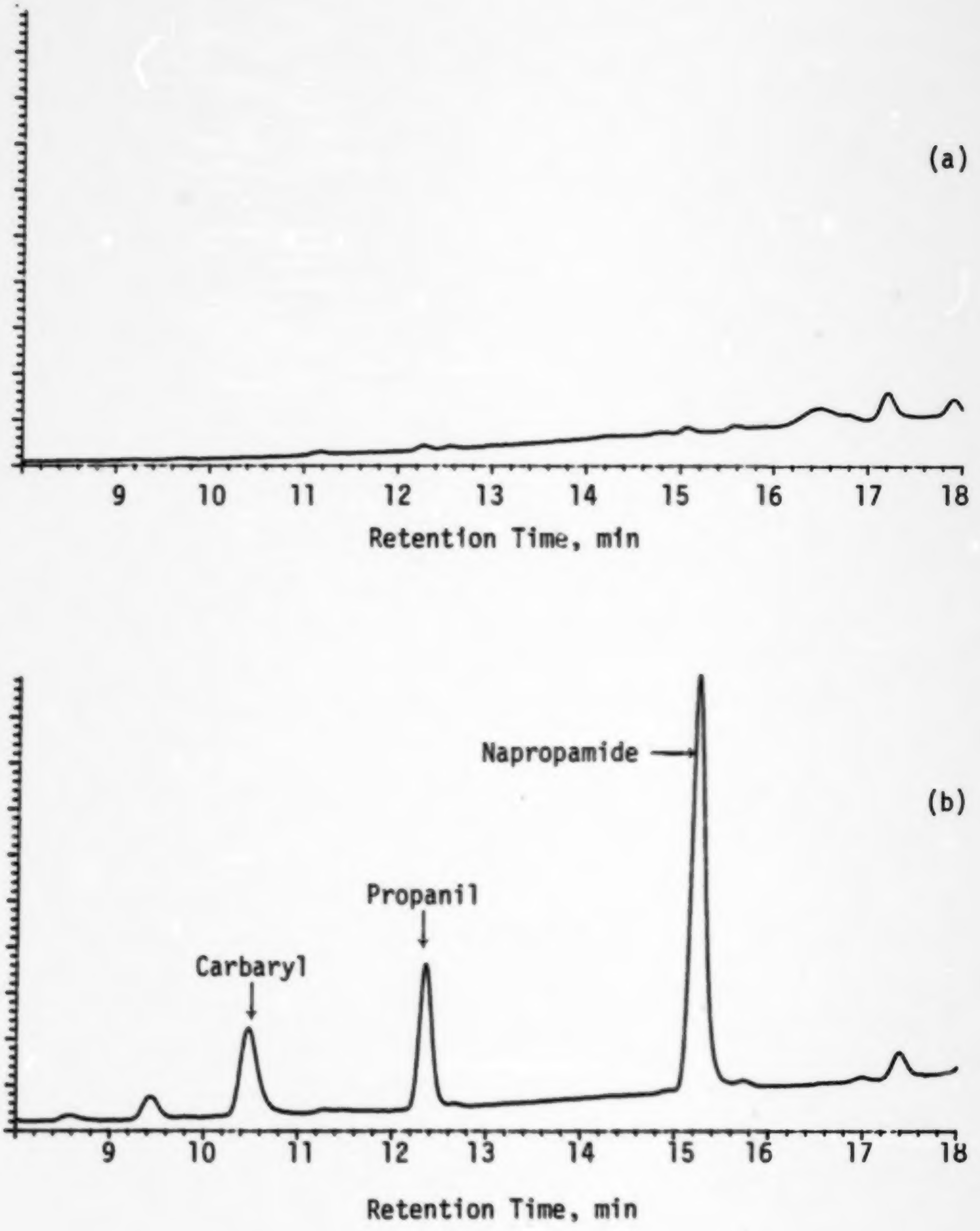


FIGURE 11. HPLC-UV CHROMATOGRAMS OF (a) REAGENT WATER, AND (b) REAGENT WATER SPIKED WITH THE CARBAMATE AND AMIDE PESTICIDES AT THE 2.0 µg/L LEVEL.

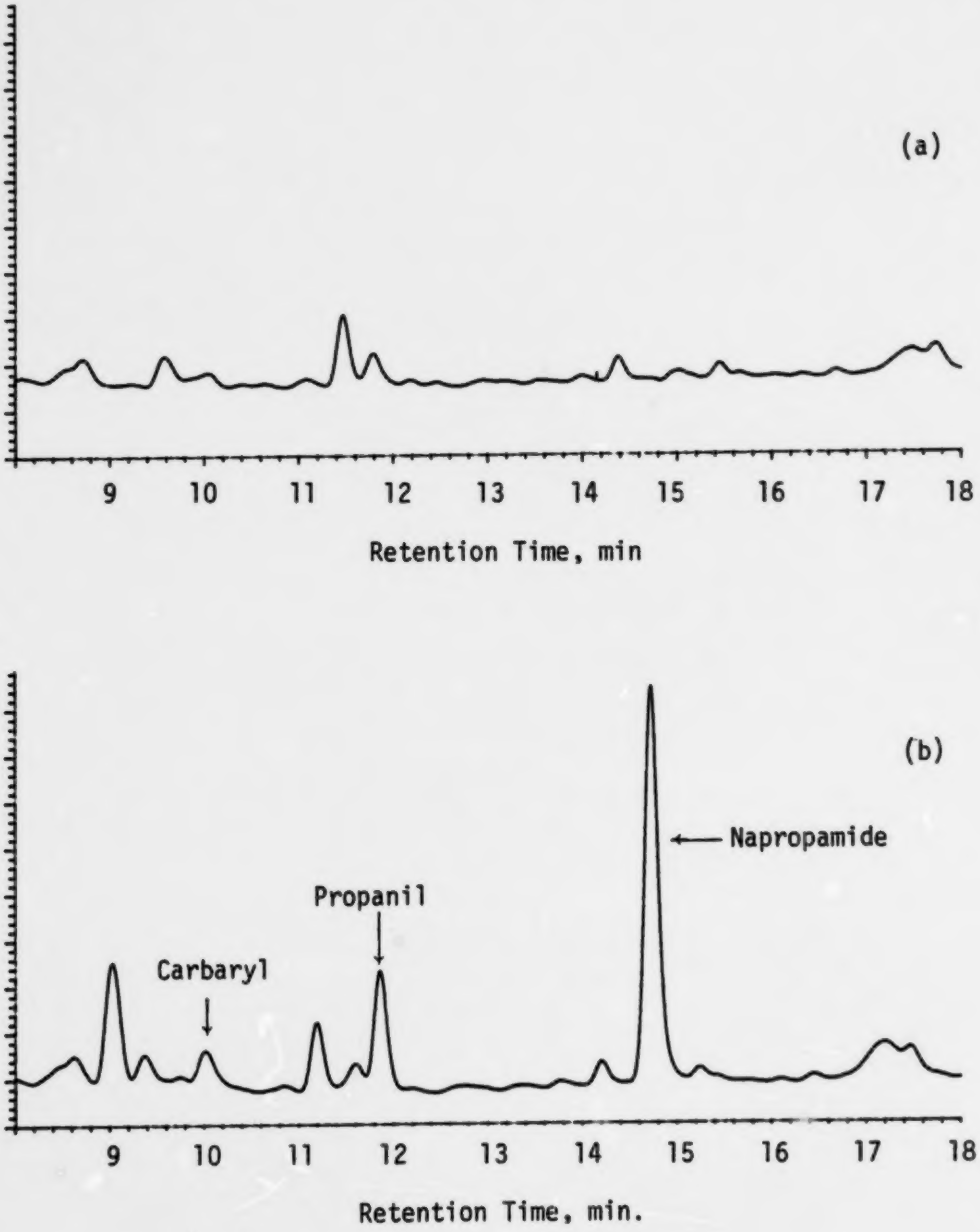


FIGURE 12. HPLC-UV CHROMATOGRAM OF (a) POTW EFFLUENT, AND (b) POTW EFFLUENT SPIKED WITH THE CARBAMATE AND AMIDE PESTICIDES AT THE 2.0 μg/L LEVEL.

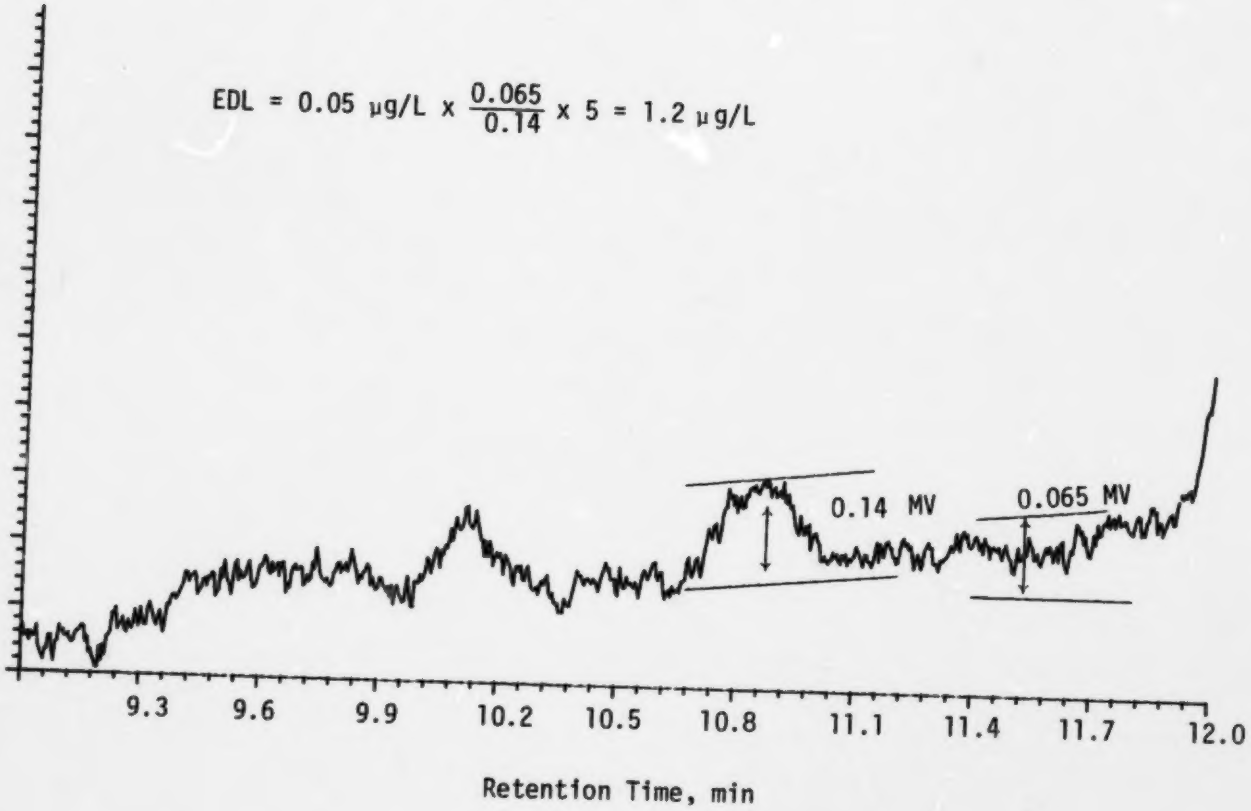
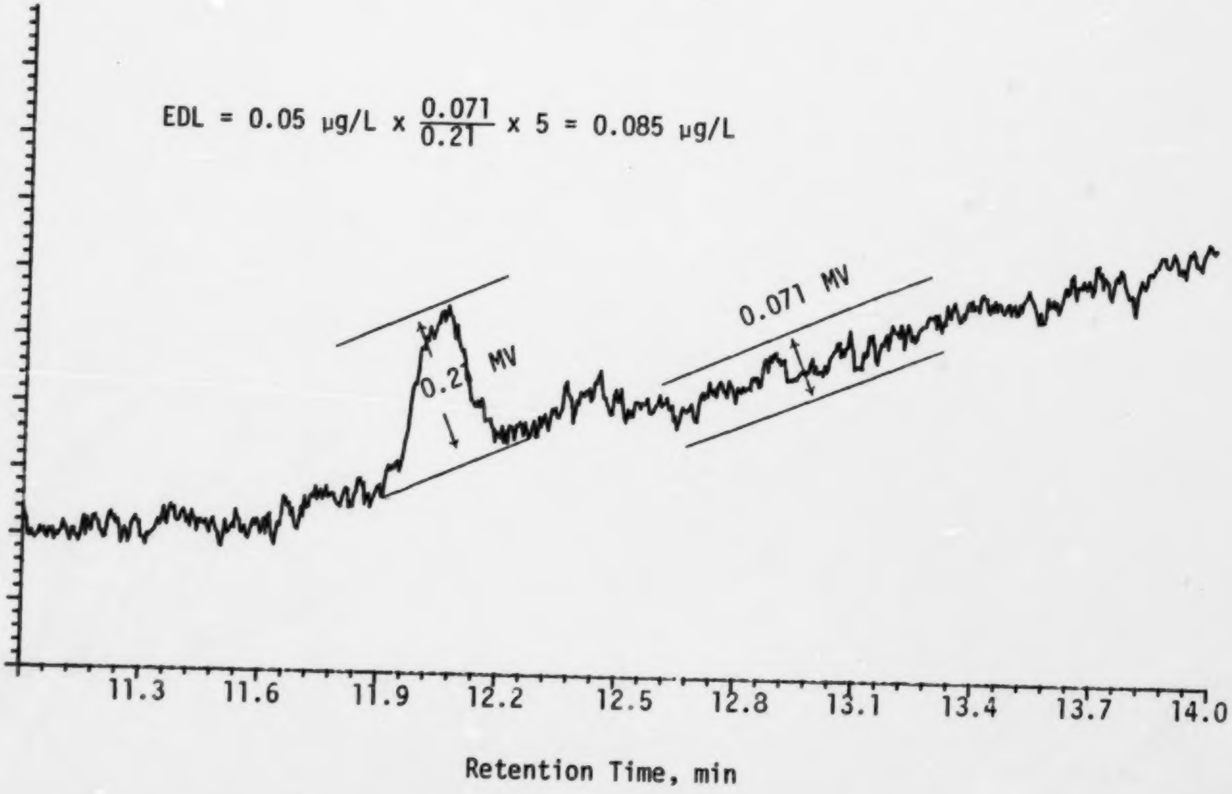


FIGURE 13. HPLC-UV CHROMATOGRAM OF CARBARYL STANDARD EQUIVALENT TO 0.5 µg/L IN WATER USED TO DETERMINE METHOD EDL.



$$EDL = 0.05\ \mu g/L \times \frac{0.071}{0.21} \times 5 = 0.085\ \mu g/L$$

FIGURE 14. HPLC-UV CHROMATOGRAM OF PROPANIL STANDARD EQUIVALENT TO 0.05 μg/L IN WATER USED TO DETERMINE METHOD EDL.

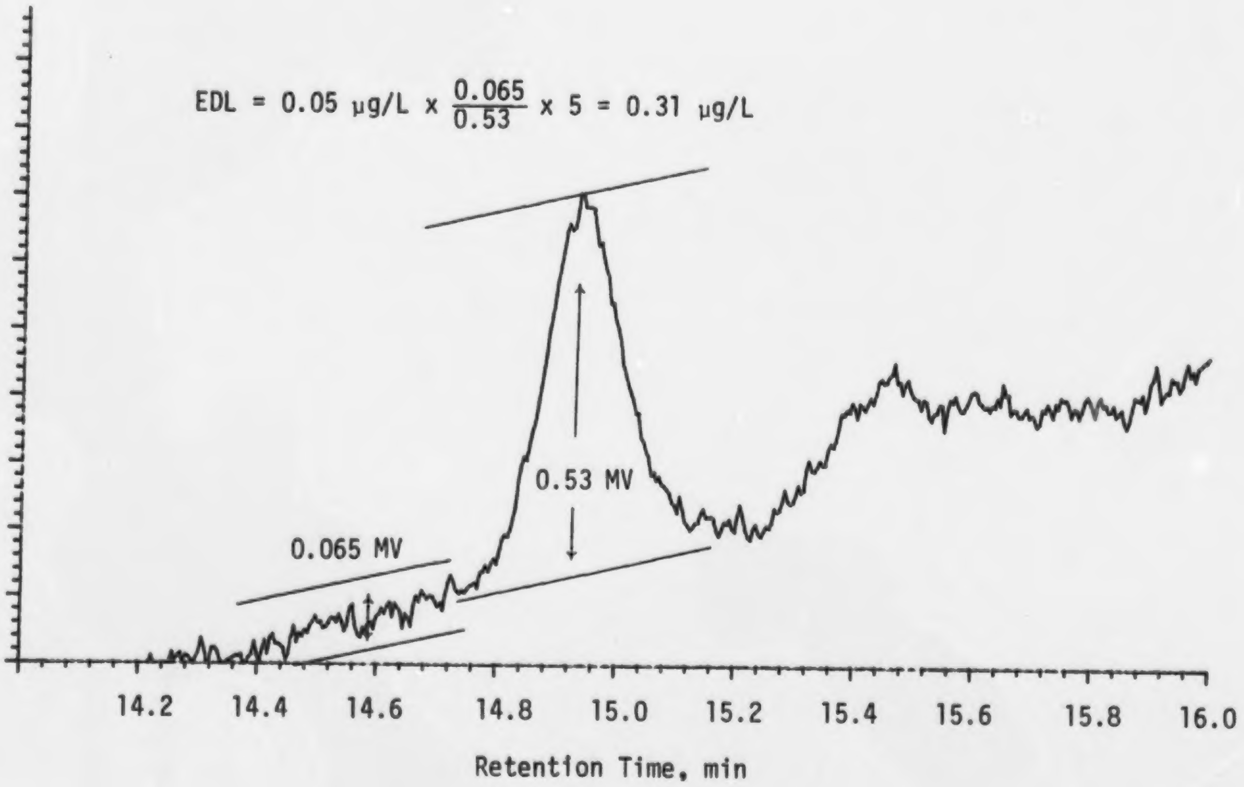


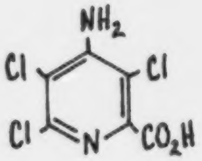
FIGURE 15. HPLC-UV CHROMATOGRAM OF NAPROPAMIDE STANDARD EQUIVALENT TO 0.5 µg/L IN WATER USED TO DETERMINE METHOD EDL.

SECTION 6

ANALYSIS OF PICLORAM IN WASTEWATER BY LIQUID CHROMATOGRAPHY

INTRODUCTION

Picloram (IX) is a systemic herbicide. The IUPAC name for picloram is 4-amino-3,5,6-trichloropyridine-2-carboxylic acid and its CAS registry number is 1918-02-1. A common synonym for picloram is Tordon. Picloram is a colorless solid which decomposes without melting at 215°C. Picloram has a solubility in water of 430 mg/L at 25°C. Picloram is acidic and forms water-soluble alkali metal and amine salts. The acute oral LD50 for rats for picloram is 8200 mg/kg (40).



IX

Analysis methods for the determination of picloram in various matrices have been reported in the literature. These methods usually involve the use of reverse-phase column HPLC coupled with a UV detector (41,42). Several analysis methods using packed or capillary column GC, usually with an electron capture detector, are also reported (43,44,45,46). These methods require a derivatization step before analysis by GC.

The method provided by the Project Officer for evaluation for determination of picloram consisted of adjustment of the sample pH to the range of 1.5 to 2.5 by addition of dilute sodium hydroxide or sulfuric acid; addition of NaCl to the sample; extraction of the sample with methylene chloride using a separatory funnel; drying of the sample using anhydrous sodium sulfate; concentration of the sample to 25 mL after solvent substitution with HPLC mobile phase; and analysis of the sample extract by reverse phase HPLC using a UV detector. A cleanup procedure is included in the method consisting of a backextraction of the picloram from the methylene chloride extract into 0.3 N NaOH.

CONCLUSIONS

A method designed to determine picloram in wastewaters was evaluated. The method was not found to be acceptable. Picloram was not recovered when spiked into reagent water at the 3.0 and 30 μg/L concentration levels. Low pilcoram recoveries, 52 to 71 percent, were achieved from POTW effluent samples spiked with picloram at the 3.0 and 30 μg/L levels.

EXPERIMENTAL

The following procedure was outlined in the picloram analysis method:

1. Place sample (approximately 1 liter) into a 2-liter separatory funnel; adjust the pH of the sample to within the range of 1.5 to 2.5 with dilute sodium hydroxide or sulfuric acid; and add 200 grams of sodium chloride.

2. Extract the sample with three 60-mL portions of methylene chloride, combining the extracts.

3. Dry the combined extracts by pouring through a chromatography column containing 10 cm of anhydrous sodium sulfate.

4. Concentrate the combined extracts to an apparent volume of 1 mL using K-D equipment with the water bath at 80 to 85°C. Add methanol to the extract, and reconcentrate the extract to an apparent volume of 0.5 mL. Transfer the concentrated extract to a 25-mL volumetric flask and dilute to volume with methanol.

5. If cleanup is required, steps 1 and 2 must be repeated with a fresh sample, and the extract from step 2 is placed in a 500-mL separatory funnel. The sample is extracted with 10 mL of 0.3 N NaOH and the aqueous extract is transferred to a 25-mL volumetric flask. The aqueous extract is adjusted to within the pH range of 1.5 to 2.5 with dilute sulfuric acid solution and diluted to volume with HPLC mobile phase.

6. Analyze the sample by HPLC with UV detection using the following conditions:
   - reverse-phase 10-micron Ultrasphere ODS, 4.5 mm ID by 250 mm column;
   - isocratic 57 percent buffer (pH 2)/43 percent methanol mobile phase;
   - flow rate of 1.0 mL/min
   - injection volume of 100 μL
   - UV detector wavelength set at 225 nm.

In consultation with the EPA Task Officer, prior to method evaluation, it was decided that a 5-micron Ultrasphere ODS column should be used instead of the 10-micron column originally specified by the method. A 10-micron Ultrasphere ODS column was not commercially available. It was also necessary to raise the temperature of the water bath to 95°C when concentrating solutions containing methanol.

Samples processed included the following:

1. Triplicate reagent water samples. Reagent water was obtained from a Millipore system.

2. Triplicate reagent water samples spiked with picloram at the 3.0 μg/L concentration level.

3. Triplicate reagent water samples spiked with picloram at the 30 μg/L concentration level.

4. Triplicate POTW secondary effluent samples. POTW secondary effluent was obtained from the City of Columbus.

5. Triplicate POTW secondary effluent samples spiked with picloram at the 3.0 μg/L concentration level.

6. Triplicate POTW secondary effluent samples spiked with picloram at the 30 μg/L concentration level.

A second set of samples, as described in points 1-6, were prepared and treated with the basic backextraction cleanup step.

Recoveries were determined by comparison to standard solutions of picloram prepared at the 0.1, 0.5, and 2.0 μg/mL concentration levels in HPLC mobile phase. These concentration levels were equivalent to 2.5, 12.5, and 50 μg/L of picloram in the original samples assuming a final extract volume of 25 mL. Response factors were calculated for the standards and used to determine concentration levels in the abovementioned reagent water and POTW effluent samples.

The method EDL, defined as the concentration of picloram in a sample yielding a S/N of 5, was determined by injecting 0.05 μg/mL solution of picloram in HPLC mobile phase.

QUALITY ASSURANCE

Instrumentation was set up as described in the method. Sensitivity achieved was approximately equivalent to that reported in the method. Calibration standards consisted of picloram solutions prepared at the 0.1, 0.5, and 25 μg/mL concentration levels in HPLC mobile phase. Each calibration standard was analyzed in duplicate prior to analysis of any evaluation samples. A selected calibration standard was then analyzed after every five evaluation samples. Response factors were calculated for each calibration run by dividing the equivalent picloram concentration level in the sample in µg/L by the picloram peak area. Resultant calibration data are reported in Table 12. Response factors were reproducible over the entire calibration range. A response factor of 0.035 was used for calculations of picloram concentrations in the evaluation samples.

TABLE 12. PICLORAM CALIBRATION DATA

Concentration, µg/L(a)	Average Peak Area	Standard Deviation	Number of Replicates	Average Response Factors
2.5	80	15	4	0.031
12.5	340	6	4	0.037
50	1390	8	4	0.036

(a) Equivalent concentration of picloram in water sample.

Standards of picloram were not obtained from two independent sources. It was not possible to check the purity of the picloram that was used for these studies by comparison to another standard.

RESULTS AND DISCUSSION

Picloram was not detected in unspiked reagent water or POTW effluent samples. The chromatograms obtained from reagent water and POTW effluent blanks contained other peaks apparently coming from the solvent used for sample and standard preparation. These peaks were not removed by the basic backextraction cleanup procedure. The peaks were not large and did not interfere with the determination of picloram. Examples of chromatograms obtained from the analyses of an aliquot of unspiked POTW effluent and POTW effluent spiked with picloram at the 3.0 µg/L level are shown in Figure 16. The basic backextraction cleanup step was not used to prepare these samples.

Recoveries of 3.0 and 30 µg/L levels of picloram from POTW effluent were low, ranging between 52 and 71 percent, both with and without the basic backextraction cleanup step. Picloram was not recovered from reagent water samples. It is not known why picloram was recovered from POTW effluent and not from reagent water. Since the spiked reagent water samples were prepared on two separate days simultaneously with spiked POTW samples, it is not likely that problems occurred during sample preparations. Since picloram recoveries were reproducibly low, regardless of whether or not the basic backextraction

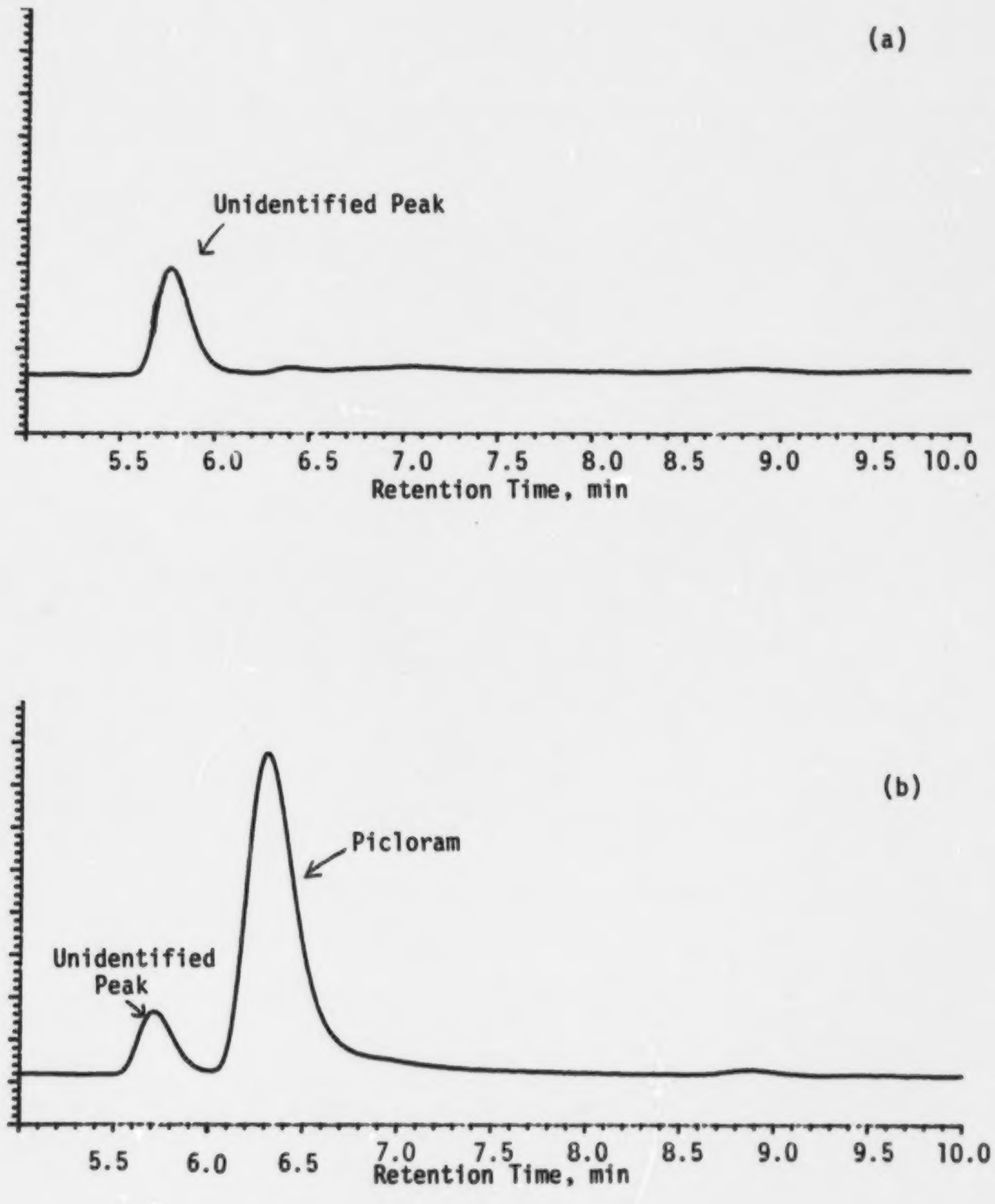


FIGURE 16. HPLC-UV CHROMATOGRAMS OF (a) POTW EFFLUENT, AND (b) POTW EFFLUENT SPIKED WITH PICLORAM AT THE 3.0 μg/L LEVEL.

cleanup step was used, recovery losses most likely occurred during the methylene chloride extraction step of the procedure. Several factors might account for low picloram recoveries. Equilibrium may not have been achieved during the extraction procedure to yield acceptable picloram recoveries using separatory funnel extraction techniques. Decomposition of the picloram in water might also account for the low recoveries. These studies did not provide enough information to suggest a particular reason for the recovery losses. Picloram recovery data are given in Table 13.

TABLE 13. RECOVERIES OF PICLORAM FROM WATER(c)

Spike Level, µg/L	Matrix (a)	With Cleanup Blank Level	With Cleanup Percent Recovery	With Cleanup Standard Deviation,%	Without Cleanup Blank Level	Without Cleanup Percent Recovery	Without Cleanup Standard Deviation,%
3.0	1	ND(b)	ND	--	ND	ND	--
30	1	ND	ND	--	ND	ND	--
3.0	2	ND	52	9	ND	58	4
30	2	ND	71	0	ND	68	3

(a) 1=Reagent water; 2=Columbus POTW secondary effluent.
(b) ND=not detected.
(c) Recovery data are averages of three replicate analyses.

The EDL for picloram was determined by analyzing a 0.05 µg/mL standard solution of picloram in HPLC mobile phase; the resultant chromatogram is shown in Figure 17. This solution yielded a S/N of 19. The EDL, defined as the concentration in water yielding a S/N equal to 5.0, was calculated to be 0.3 µg/L of picloram. The EDL does not take into account any interferences or recovery losses that might be encountered from a particular matrix.

RECOMMENDATIONS

The method was not acceptable for determination of picloram in reagent water and POTW effluents. The HPLC-UV analysis procedure proved to be sensitive and repeatable. However, recoveries of picloram from the two water matrices were very low, presumably due to picloram decomposition or difficulties encountered in extracting the picloram from the water with methylene chloride. Picloram stability in water should be studied over a two or three day period to see if picloram concentrations remain constant.

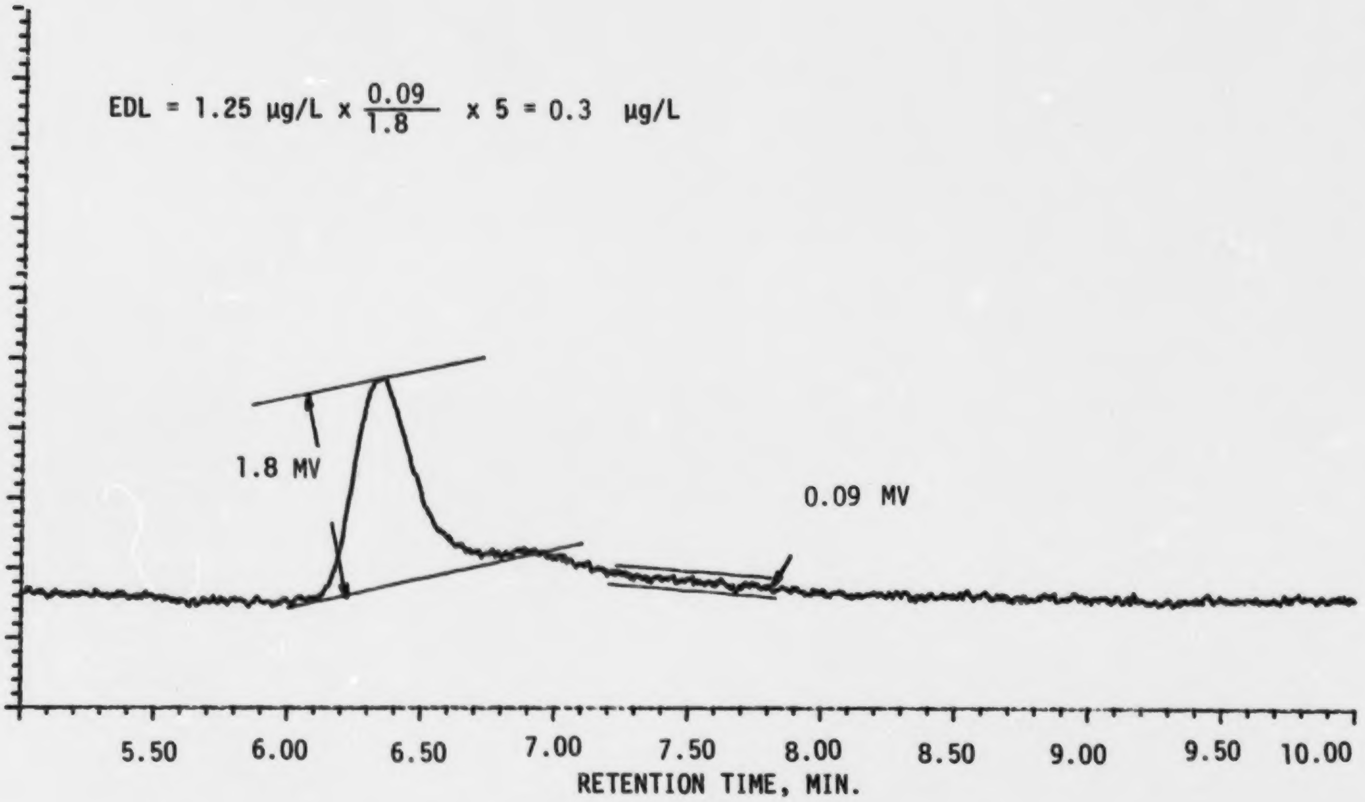


FIGURE 17. HPLC-UV CHROMATOGRAM OF PICLORAM STANDARD EQUIVALENT TO 1.25 μg/L IN WATER USED TO DETERMINE METHOD EDL.

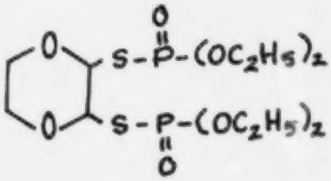
The extraction step of this procedure should be examined to determine possible means to improve picloram extraction efficiencies. In order to improve extraction efficiencies, other extraction methods should be examined. Continuous liquid-liquid extraction or tumbling might improve recoveries by assuring optimum mixing of the two phases; success of separatory funnel extractions are often operator dependent because the separatory funnel is not shaken vigorously or long enough.

SECTION 7

ANALYSIS OF ORGANOPHOSPHORUS PESTICIDES IN WASTEWATER BY GAS CHROMATOGRAPHY

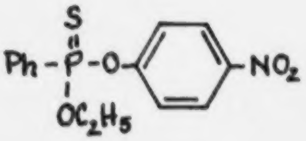
INTRODUCTION

Dioxathion (X) is a non-systemic insecticide and acaricide. The IUPAC name for dioxathion is S,S'-(1,4-dioxane-2,3-diyl)O,O,O',O'-tetraethyl di(phosphorodithioate) and its CAS registry number is 78-34-2. A common synonym for dioxathion is Delnav. Technical dioxathion is a brown liquid which is insoluble in water and soluble in most organic solvents. Dioxathion is stable in water but is hydrolyzed by alkali or on heating. The acute oral LD50 for rats for dioxathion is 43 mg/kg (47).



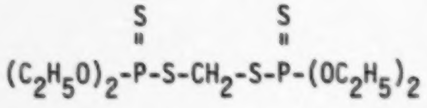
X

EPN (XI) is a non-systemic insecticide and acaricide. The IUPAC name for EPN is O-ethyl O-4-nitrophenyl phenylphosphonothioate and its CAS registry number is 2104-64-5. EPN is a light yellow crystalline powder with a melting point of 36°C. EPN is practically insoluble in water and soluble in most organic solvents. EPN is stable in neutral and acidic media but is hydrolyzed by alkali. The acute oral LD50 for rats for EPN is 33-42 mg/kg (48).



XI

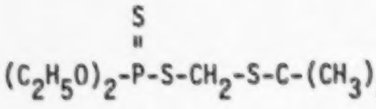
Ethion (XII) is a non-systemic insecticide and acaricide. The IUPAC name for ethion is O,O,O',O'-tetraethyl S,S'-methylene di(phosphorodithioate) and its CAS registry number is 563-12-2. Ethion is a colorless to amber-colored liquid. Ethion is sparingly soluble in water and soluble in most organic solvents. Ethion is slowly oxidized in air and is hydrolyzed by acids and alkali. The acute oral LD50 for rats for ethion is 208 mg/kg (49).



$$(C_2H_5O)_2\text{-}\overset{\overset{S}{\|}}{P}\text{-}S\text{-}CH_2\text{-}S\text{-}\overset{\overset{S}{\|}}{P}\text{-}(OC_2H_5)_2$$

XII

Terbufos (XIII) is an insecticide. The IUPAC name for terbufos is S-tert-butylthiomethyl O,O-diethyl phosphordithioate and its CAS registry number is 13071-79-9. A common synonym for terbufos is Counter. Terbufos is a colorless to pale yellow liquid. The solubility of terbufos in water is 10-15 mg/L at room temperature and it is soluble in most organic solvents. Terbufos decomposes at temperatures greater than 120°C or in the presence of acids and alkali. The acute oral LD50 for rats for terbufos is 1.6 mg/kg (50).



$$(C_2H_5O)_2\text{-}\overset{\overset{S}{\|}}{P}\text{-}S\text{-}CH_2\text{-}S\text{-}C\text{-}(CH_3)_2$$

XIII

Analysis methods for the determination of the organophosphorus pesticides in various matrices have been reported in the literature. These methods use packed or capillary column GC with various detectors such as alkali flame, flame photometric, electron capture, and mass spectrometric detectors (24,38,51-66).

The method provided by the Project Officer for evaluation for determination of organophosphorus pesticides consisted of adjustment of the sample pH to the range of 5 to 9 by addition of dilute sodium hydroxide or sulfuric acid; extraction of the sample with 15 percent methylene chloride in hexane using a separatory funnel; drying of the sample using anhydrous sodium sulfate; concentration of the sample to 1 mL; and analysis of the sample extract by packed column GC-NPD. A cleanup procedure using 3 percent deactivated silica gel was included in the method.

CONCLUSIONS

A method designed to determine organophosphorus pesticides in wastewaters was evaluated. The extraction and analysis portions of the method were found to be acceptable for the determinations of dioxathion, EPN, ethion, and terbufos in reagent water and POTW effluent; recoveries of these compounds spiked into these matrices at the 10 and 100 µg/L levels were generally greater than 80 percent. The silica gel cleanup procedure was not acceptable; recoveries of dioxathion, EPN, ethion, and terbufos were decreased by 50 percent or more when the cleanup procedure was used.

EXPERIMENTAL

The following procedure was outlined in the organophosphorus pesticide analysis method:

1. Place sample (approximately 1 liter) into a 2-liter separatory funnel, and adjust the pH of the sample to within the range of 5 to 9 with dilute sodium hydroxide or sulfuric acid.

2. Extract the sample with three 60-mL portions of 15 percent methylene chloride in hexane and combine the extracts.

3. Dry the combined extracts by pouring through a chromatography column containing 10 cm of anhydrous sodium sulfate.

4. Concentrate the combined extracts to 1 mL using K-D equipment with the water bath at 90 to 95°C.

5. A cleanup procedure using 3 percent deactivated silica gel is used for samples that require cleanup. Silica gel, 10 grams, is slurried in hexane and placed in a chromatography column. The sample is applied to the column in hexane, and the column is eluted with 30 mL of 6 percent methylene chloride in hexane and 30 mL of 15 percent methylene chloride in hexane. The eluates are discarded. The column is then eluted with 30 mL of 50 percent methylene chloride in hexane (F1) and 30 mL of methylene chloride (F2). Terbufos and ethion elute in F1. Dioxathion and EPN elute in F1 and F2. Each fraction is concentrated to 1 mL using K-D equipment with the water bath at 90 to 95°C. A hexane solvent exchange step is included.

6. Analyze the sample by GC-NPD using the following conditions:
   - 180 cm long by 2 mm ID glass column packed with 3 percent OV-225 on 100/120 mesh Gas-Chrom Q;
   - nitrogen carrier gas at 50 mL/min flow rate;
   - oven temperature of 200°C isothermal for 2 min, programmed to 240°C at 4°C/min, held at 240°C for 8 min;
   - injection volume of 5 µL.

Samples analyzed included the following:

1. Triplicate reagent water samples.

2. Triplicate reagent water samples spiked with the organophosphorus pesticides at the 10 μg/L concentration level.

3. Triplicate reagent water samples spiked with the organophosphorus pesticides at the 100 μg/L concentration level.

4. Triplicate POTW secondary effluent samples. POTW secondary effluent was obtained from the City of Columbus.

5. Triplicate POTW secondary effluent samples spiked with the organophosphorus pesticides at the 10 μg/L concentration level.

6. Triplicate POTW secondary effluent samples spiked with the organophosphorus pesticides at the 100 μg/L concentration level.

All extracts described in points 1-6 were analyzed and then treated to the silica gel cleanup procedure and reanalyzed by GC-NPD.

Recoveries were determined by comparison to standard solutions of the organophosphorus pesticides prepared at the 0.5, 1.0, 5.0, 7.0, 15, and 20 μg/mL concentration levels in hexane. These concentration levels were equivalent to 0.5, 1.0, 5.0, 7.0, 15, and 20 μg/L of the organophosphorus pesticides in the original samples assuming a final extract volume of 1 mL. Response factors were calculated for the standards and used to determine concentration levels in the abovementioned reagent water and POTW effluent samples.

The method EDLs, defined as the concentration of each organophosphorus pesticide in a sample yielding a S/N of 5, were determined using two different NPD rubidium beads. EDLs were determined for the first bead by injecting 0.5, 0.5, 0.1, and 0.01 μg/mL solutions of dioxathion, EPN, ethion, and terbufos in hexane, respectively. EDLs were determined for the second bead by injecting 1.0, 7.0, 0.5, and 0.2 μg/mL solutions of dioxathion, EPN, ethion, and terbufos in hexane, respectively.

QUALITY ASSURANCE

Instrumentation was set up as described in the method. Sensitivity achieved was significantly better than that described in the method for dioxathion, ethion, and terbufos, and approximately equal to that described for EPN. Detector sensitivity, however, was found to be extremely dependent upon the bead used in the detector. When a bead was replaced in the NPD, detector sensitivity for these compounds was decreased by factors of 3 to 250. Calibration standards used to analyze the samples before cleanup were prepared containing dioxathion, EPN, ethion, and terbufos at the 0.5, 1.0, and 5.0 μg/mL concentration levels. These are equivalent to compound concentrations of 0.5, 1.0, and 5.0 µg/L in a water sample. Sample extracts were diluted by factors of 10 or 20 to bring their final concentrations to within this calibration range. Calibration standards used to analyze the samples after cleanup were prepared containing dioxathion, EPN, ethion, and terbufos at the 7.0, 15, and 20 µg/mL concentration levels. These are equivalent to compound concentrations of 7.0, 15, and 20 µg/L in a water sample. Sample extracts were diluted, if necessary, by a factor of 10 to bring their final concentrations to within this calibration range. These calibration standards were analyzed in duplicate prior to analyses of the water extracts; a calibration standard was analyzed after every five water extracts. Response factors were calculated for each calibration run by dividing the concentration level in µg/L by the corresponding peak area. Resultant calibration data are reported in Tables 14 and 15. Response factors were repeatable over the entire calibration range. Response factors of 0.017, 0.0032, 0.0014, and 0.0018 were used for dioxathion, EPN, ethion, and terbufos concentration determinations before cleanup, respectively. Response factors of 0.022, 0.013, 0.0026, and 0.0028 were used for dioxathion, EPN, ethion, and terbufos concentration determinations after cleanup, respectively.

Standards of dioxathion, EPN, ethion, and terbufos were obtained from two independent sources. Calibration standards used for quantification of these compounds in the water samples were prepared from compounds obtained from the reference standards repository of EPA-HERL. A test standard was prepared containing each of the compounds at the 5.0 µg/mL concentration level in hexane using compounds obtained from a chemical supply house. This test calibration standard was analyzed in triplicate and the resultant peak areas were compared to those generated from analyses of the original calibration standards. Resultant data indicated that for dioxathion, ethion, and terbufos, the standard obtained from the chemical supply house was not as pure as that obtained from the reference standard repository. Peak areas obtained from the test calibration standards of dioxathion, ethion, and terbufos were 12 percent, 17 percent, and 21 percent lower than those obtained from the original calibration standards, respectively. However, peak areas obtained from the test calibration standards of EPN were 8.6 percent higher than those obtained from the original calibration standards.

RESULTS AND DISCUSSION

Prior to the silica gel cleanup step, low levels of terbufos or a compound coeluting with terbufos, were detected in the reagent water and the POTW effluent. Low levels of dioxathion or a compound coeluting with dioxathion were also detected in the POTW effluent. The presence of these interferences did not hinder determination of any of the organophosphorus pesticides. Example chromatograms from the analyses of reagent water and POTW effluent, unspiked and spiked with the organophosphorus pesticides at the 100 µg/L levels are shown in Figures 18 and 19. The samples displayed in Figures 18 and 19 were not subjected to the silica gel cleanup step.

Recoveries of the organophosphorus pesticides from water were generally greater than 80 percent, indicating that the extraction and analysis procedure

TABLE 14. ORGANOPHOSPHORUS PESTICIDES CALIBRATION DATA (BEFORE CLEANUP)

Compound	Concentration, ug/L (a)	Average Peak Area	Standard Deviation, %	Number of Replicates	Average Response Factors
Dioxathion	0.5	27.9	5	4	0.018
Dioxathion	1.0	56.9	3	4	0.018
Dioxathion	5.0	311	1	3	0.016
EPN	0.5	134	11	4	0.0037
EPN	1.0	320	8	4	0.0031
EPN	5.0	1940	4	3	0.0026
Ethion	0.5	358	4	4	0.0014
Ethion	1.0	773	4	4	0.0013
Ethion	5.0	3560	3	3	0.0014
Terbufos	0.5	295	3	4	0.0017
Terbufos	1.0	591	2	4	0.0017
Terbufos	5.0	2490	3	3	0.0020

(a) Equivalent concentration of compound in water sample.

TABLE 15. ORGANOPHOSPHORUS PESTICIDES CALIBRATION DATA (AFTER CLEANUP)

Compound	Concentration, ug/L(a)	Average Peak Area	Standard Deviation, %	Number of Replicates	Average Response Factors
Dioxathion	7.0	287	1	4	0.024
Dioxathion	15	727	4	4	0.021
Dioxathion	20	1030	3	4	0.019
EPN	7.0(b)	139	23	4	0.050
EPN	15	1030	4	4	0.015
EPN	20	1830	3	4	0.011
Ethion	7.0	2430	3	4	0.0029
Ethion	15	5950	3	4	0.0025
Ethion	20	8080	3	4	0.0025
Terbufos	7.0	2730	3	4	0.0026
Terbufos	15	5210	2	4	0.0029
Terbufos	20	6610	2	4	0.0030

(a) Equivalent concentration of compound in water sample.
(b) Concentration close to the EDL for EPN; data point not used for determination of response factor.

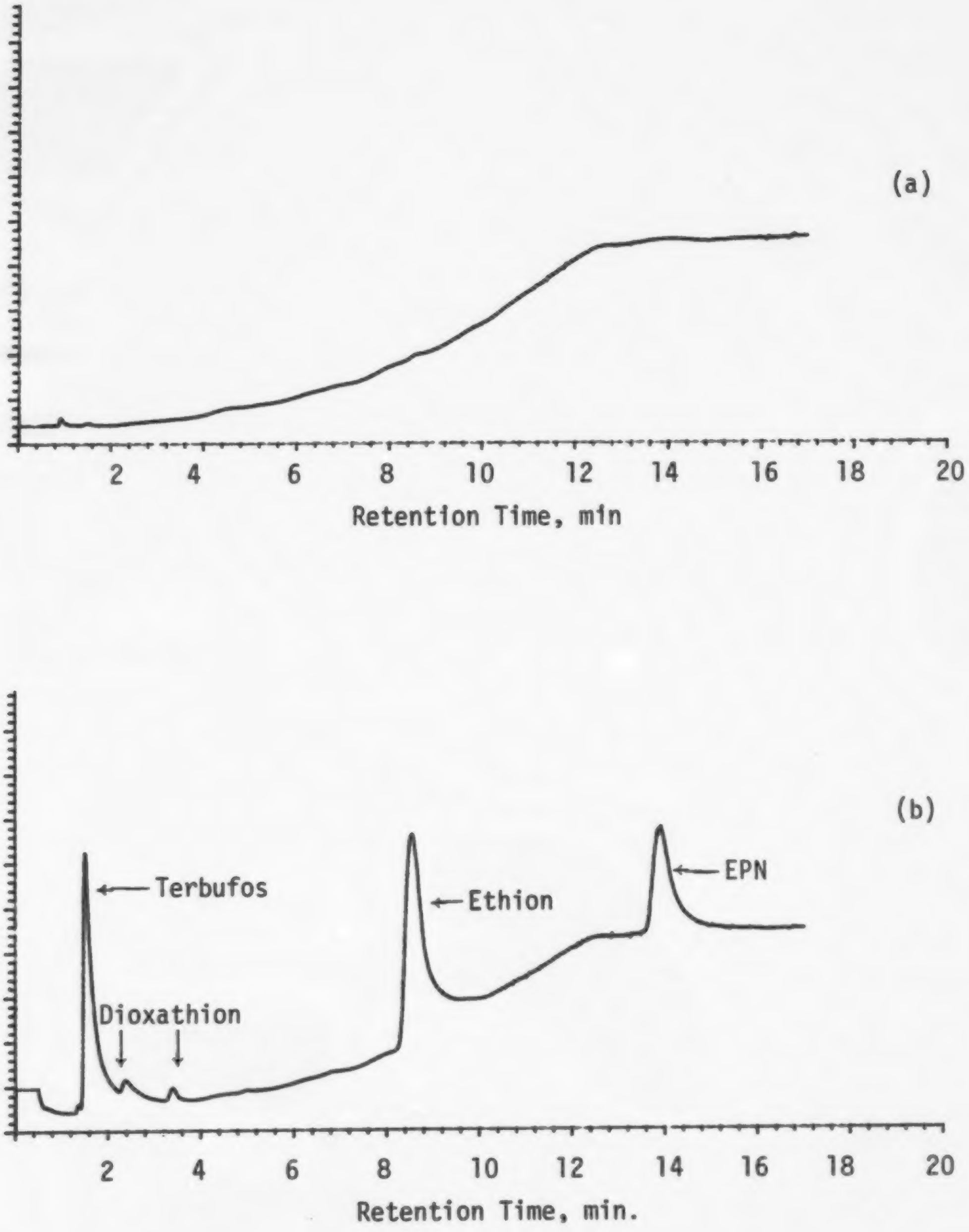


FIGURE 18. GC-NPD CHROMATOGRAM OF (a) REAGENT WATER, AND (b) REAGENT WATER SPIKED WITH THE ORGANOPHOSPHORUS PESTICIDES AT THE 100 μg/L LEVEL.

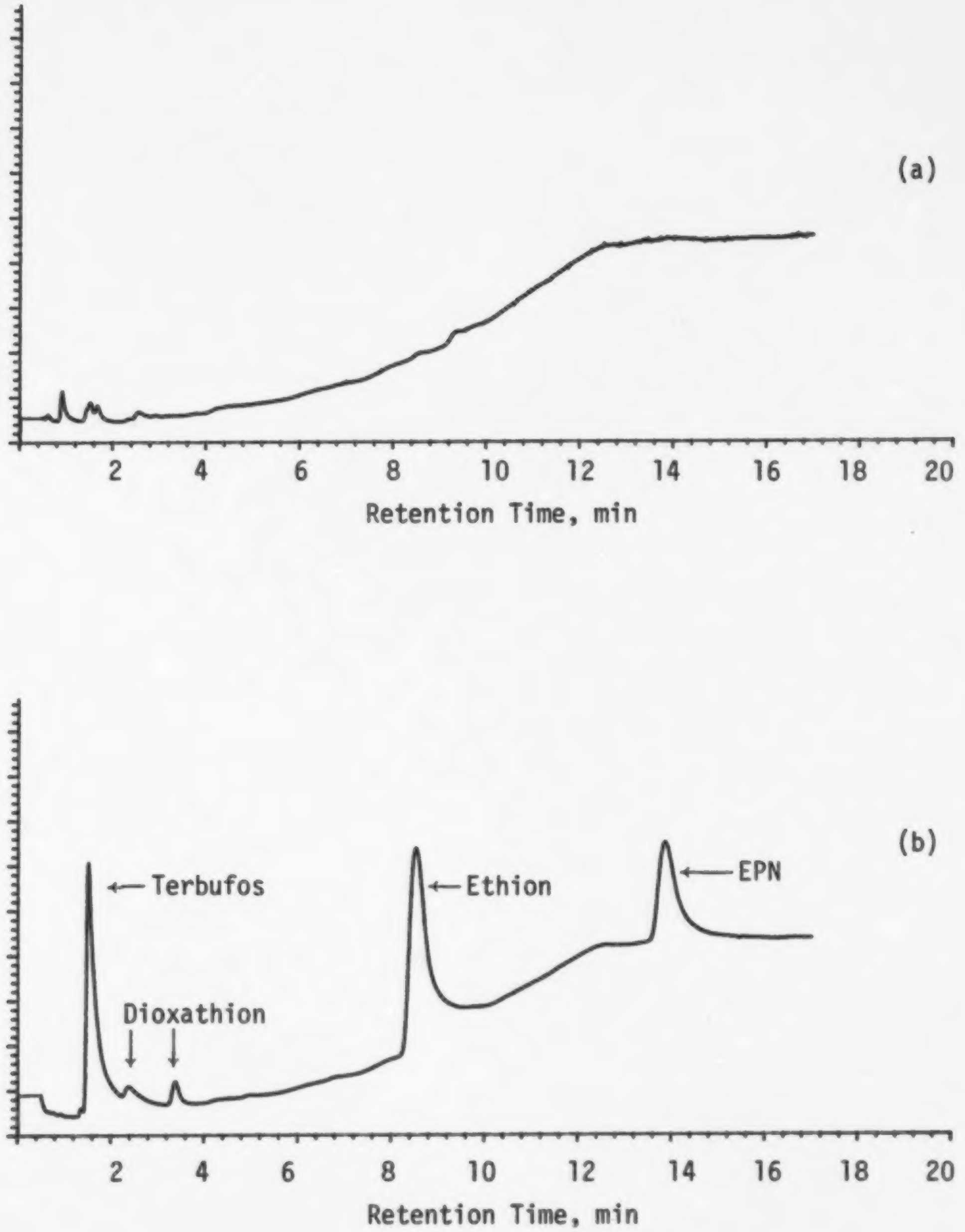


FIGURE 19. GC-NPD CHROMATOGRAM OF (a) POTW EFFLUENT, AND (b) POTW EFFLUENT SPIKED WITH THE ORGANOPHOSPHORUS PESTICIDES AT THE 100 μg/L LEVEL.

specified was effective. However, when the silica gel cleanup procedure was applied to the samples, recoveries of the organophosphorus pesticides were decreased by at least half. In some cases, the compound was no longer detected in the sample extract. The silica gel cleanup procedure was not acceptable for the determination of these compounds due to poor recoveries. These recovery data are given in Table 16.

The sensitivity of the method, and therefore the method EDLs, is dependent upon the detector sensitivity. The NPD sensitivity varies with the condition of the detector bead. In most cases, installing and conditioning a new bead does not assure maximum detector sensitivity. This was demonstrated when the method EDLs were determined on two occasions. The first set of EDLs were determined with an old bead which was very sensitive. The second set of EDLs was determined after the bead had been replaced with a new bead which was conditioned prior to use. The EDLs were 3 to 250 times higher with the new bead. These EDL data are shown in Table 17. Chromatograms used to determine these EDLs are given in Figures 20-27.

RECOMMENDATIONS

The extraction and analysis procedure for the determination of the organophosphorus pesticides is acceptable. However, the cleanup procedure using 3 percent deactivated silica gel is not acceptable. Recoveries of the organophosphorus compounds from the silica gel were poor as determined from the analyses of F1 and F2 for all four pesticides. Possibly some of the organophosphorus compounds might have eluted in the initial column washes which were discarded before analysis. Poor recoveries are more likely due to reversible or irreversible adsorption to the silica gel.

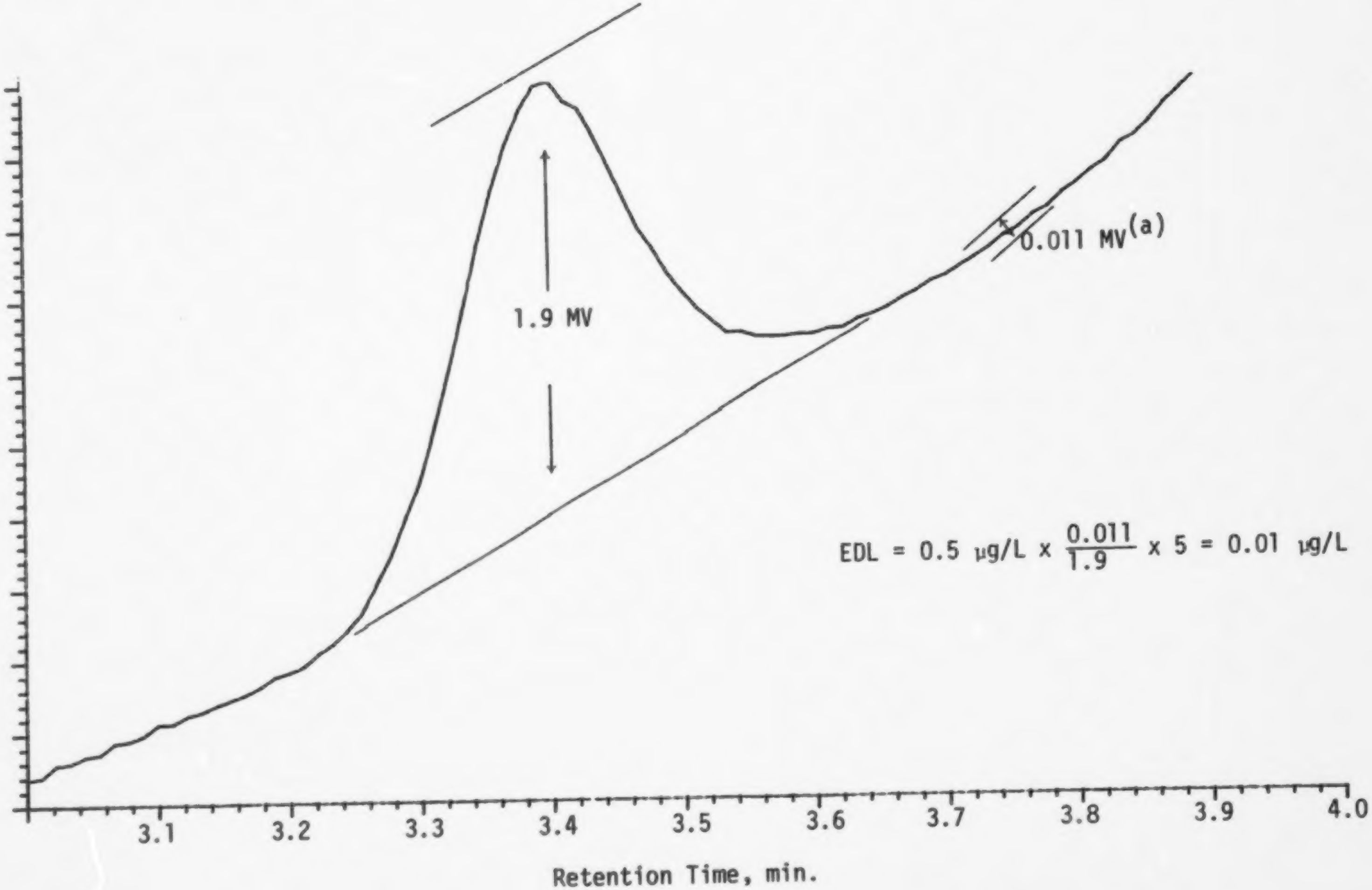
TABLE 16. RECOVERIES OF ORGANOPHOSPHORUS PESTICIDES FROM WATER(c)

Compound	Spike Level, µg/L	Matrix(a)	Before Cleanup			After Cleanup		
			Blank Level	Recovery, %	Standard Deviation,%	Blank Level	Recovery, %	Standard Deviation,%
Dioxathion	10	1	ND(b)	76	10	ND	43	7
Dioxathion	100	1	ND	78	2	ND	58	6
Dioxathion	10	2	1.6	87	15	ND	67	6
Dioxathion	100	2	1.6	91	3	ND	74	3
EPN	10	1	ND	120	6	ND	ND	-
EPN	100	1	ND	120	4	ND	65	14
EPN	10	2	ND	85	2	ND	ND	-
EPN	100	2	ND	110	6	ND	62	4
Ethion	10	1	ND	120	5	ND	54	8
Ethion	100	1	ND	95	2	ND	78	11
Ethion	10	2	ND	94	5	ND	59	12
Ethion	100	2	ND	86	5	ND	79	3
Terbufos	10	1	0.019	90	3	ND	ND	-
Terbufos	100	1	0.019	84	1	ND	42	13
Terbufos	10	2	0.21	94	5	0.13	57	4
Terbufos	100	2	0.21	77	3	0.13	54	8

(a) 1=Reagent water; 2=Columbus POTW secondary effluent.
(b) ND=not detected.
(c) Recovery data are averages of three replicate analyses.

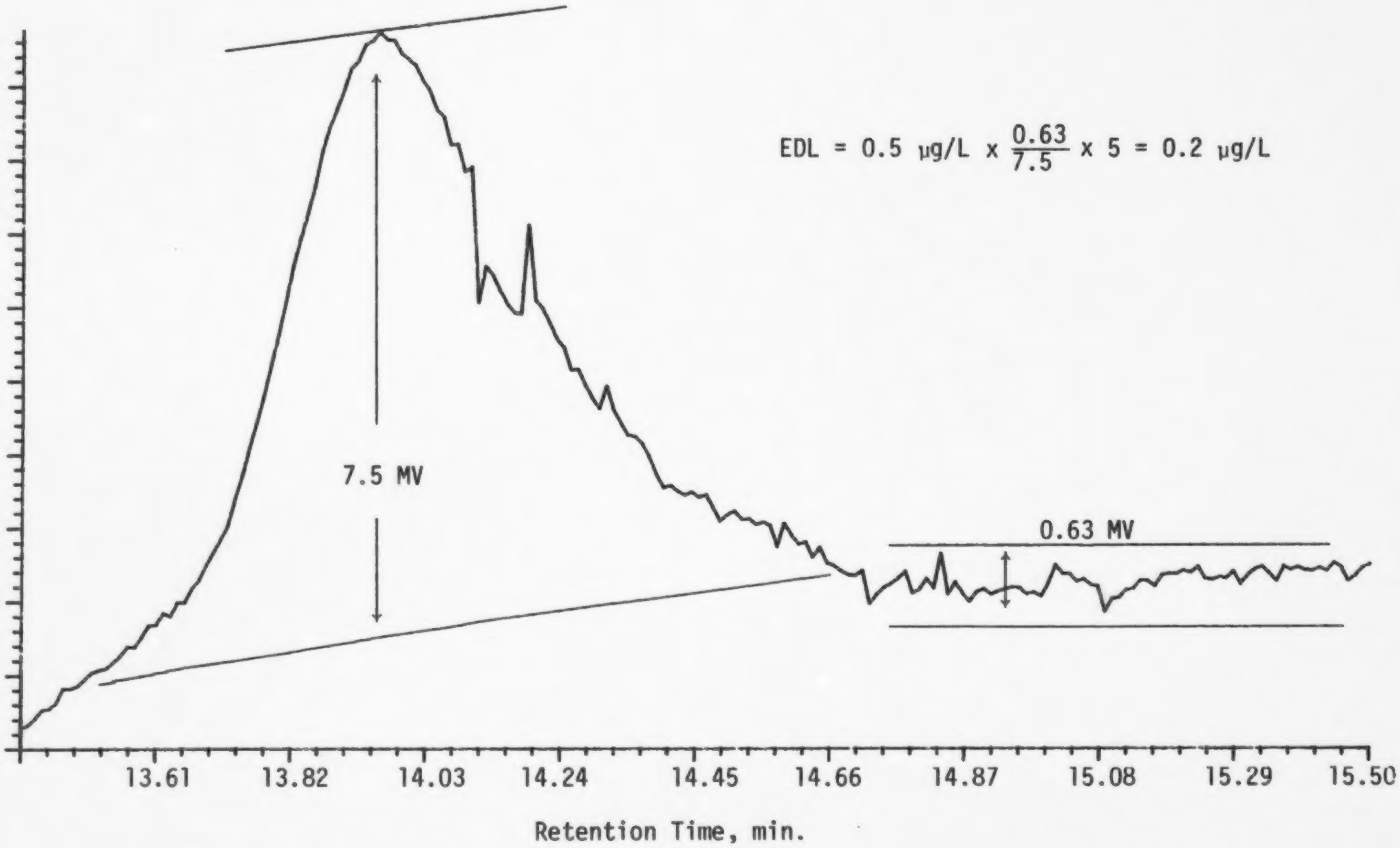
TABLE 17. ORGANOPHOSPHORUS PESTICIDES EDLs

Compound	Estimated Detection Limit, µg/L	
	Bead 1	Bead 2
Dioxathion	0.01	2.5
EPN	0.2	12
Ethion	0.09	0.3
Terbufos	0.004	0.02



$$EDL = 0.5\ \mu g/L \times \frac{0.011}{1.9} \times 5 = 0.01\ \mu g/L$$

FIGURE 20. GC-NPD CHROMATOGRAM (USING BEAD 1) OF DIOXATHION STANDARD EQUIVALENT TO 0.5 μg/L IN WATER USED TO DETERMINE METHOD EDL. (a) BASELINE DETERMINED FROM EXPANDED CHROMATOGRAM.



$$\mathrm{EDL} = 0.5\ \mu g/L \times \frac{0.63}{7.5} \times 5 = 0.2\ \mu g/L$$

FIGURE 21. GC-NPD CHROMATOGRAM (USING BEAD 1) OF EPN STANDARD EQUIVALENT TO 0.5 μg/L IN WATER USED TO DETERMINE METHOD EDL.

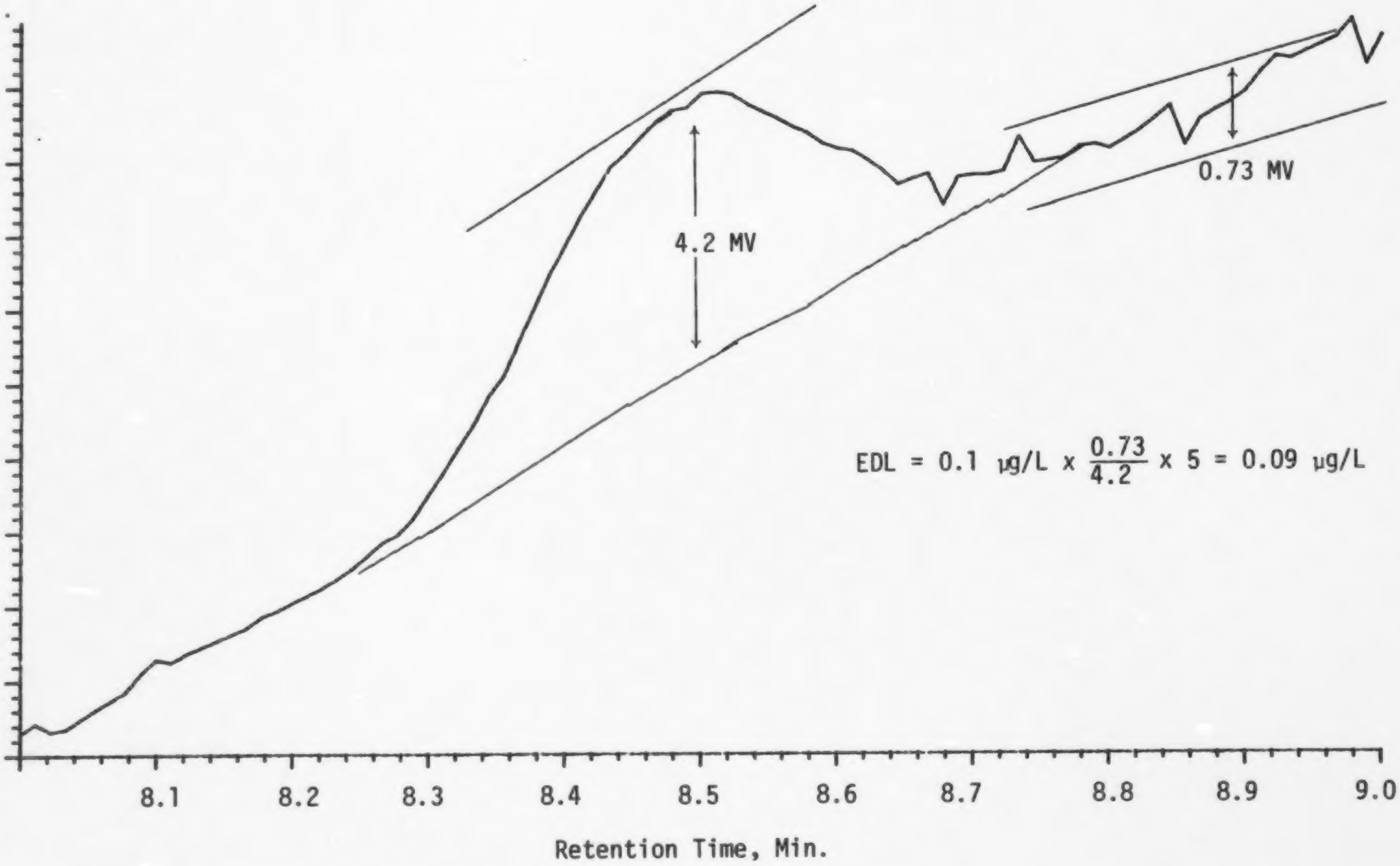


FIGURE 22. GC-NPD CHROMATOGRAM (USING BEAD 1) OF ETHION STANDARD EQUIVALENT TO 0.1 µg/L IN WATER USED TO DETERMINE METHOD EDL.

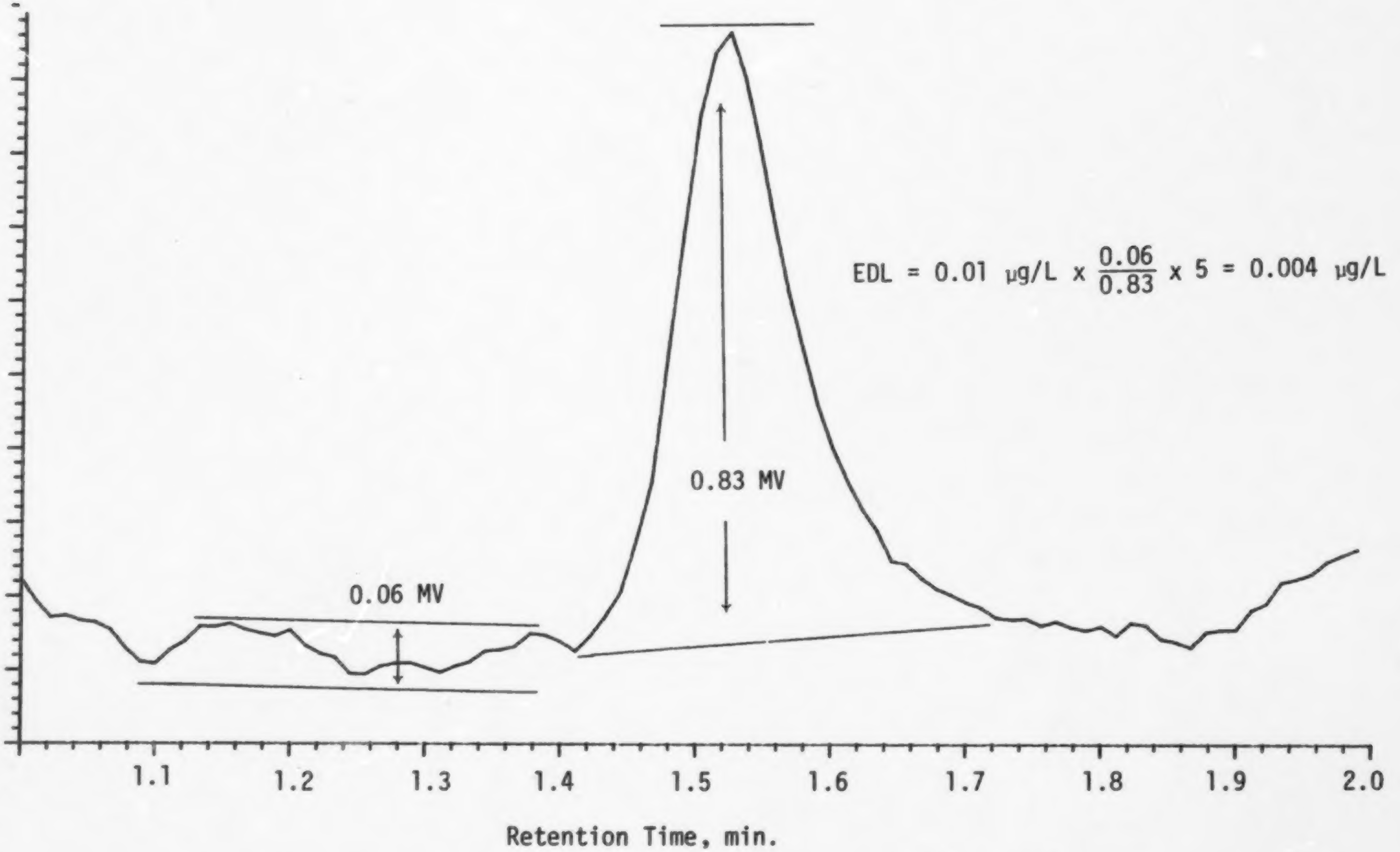
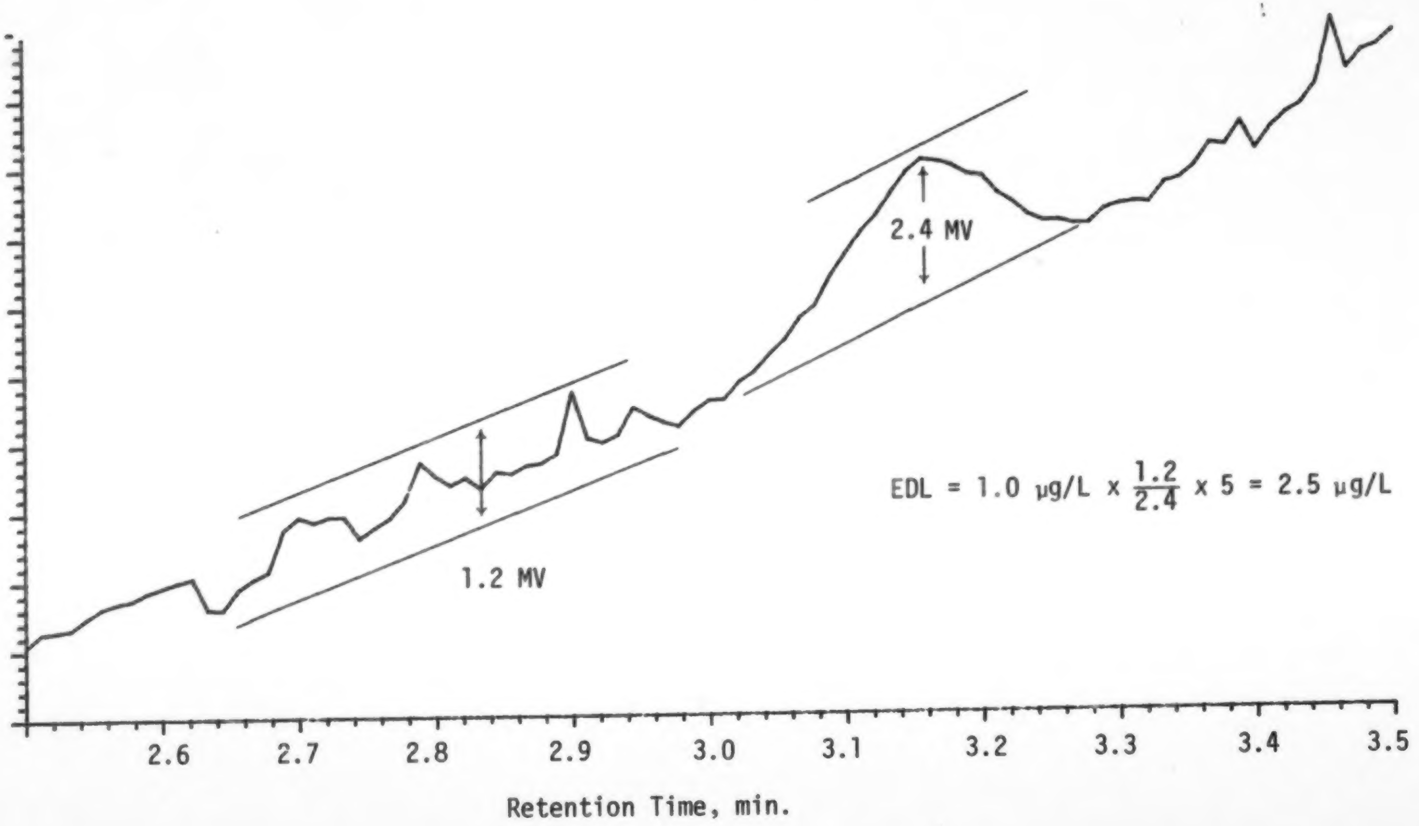


FIGURE 23. GC-NPD CHROMATOGRAM (USING BEAD 1) OF TERBUFOS STANDARD EQUIVALENT TO 0.1 µg/L IN WATER USED TO DETERMINE METHOD EDL.



$$\text{EDL} = 1.0\ \mu\text{g/L} \times \frac{1.2}{2.4} \times 5 = 2.5\ \mu\text{g/L}$$

FIGURE 24. GC-NPD CHROMATOGRAM (USING BEAD 2) OF DIOXATHION STANDARD EQUIVALENT TO 1.0 µg/L IN WATER USED TO DETERMINE METHOD EDL.

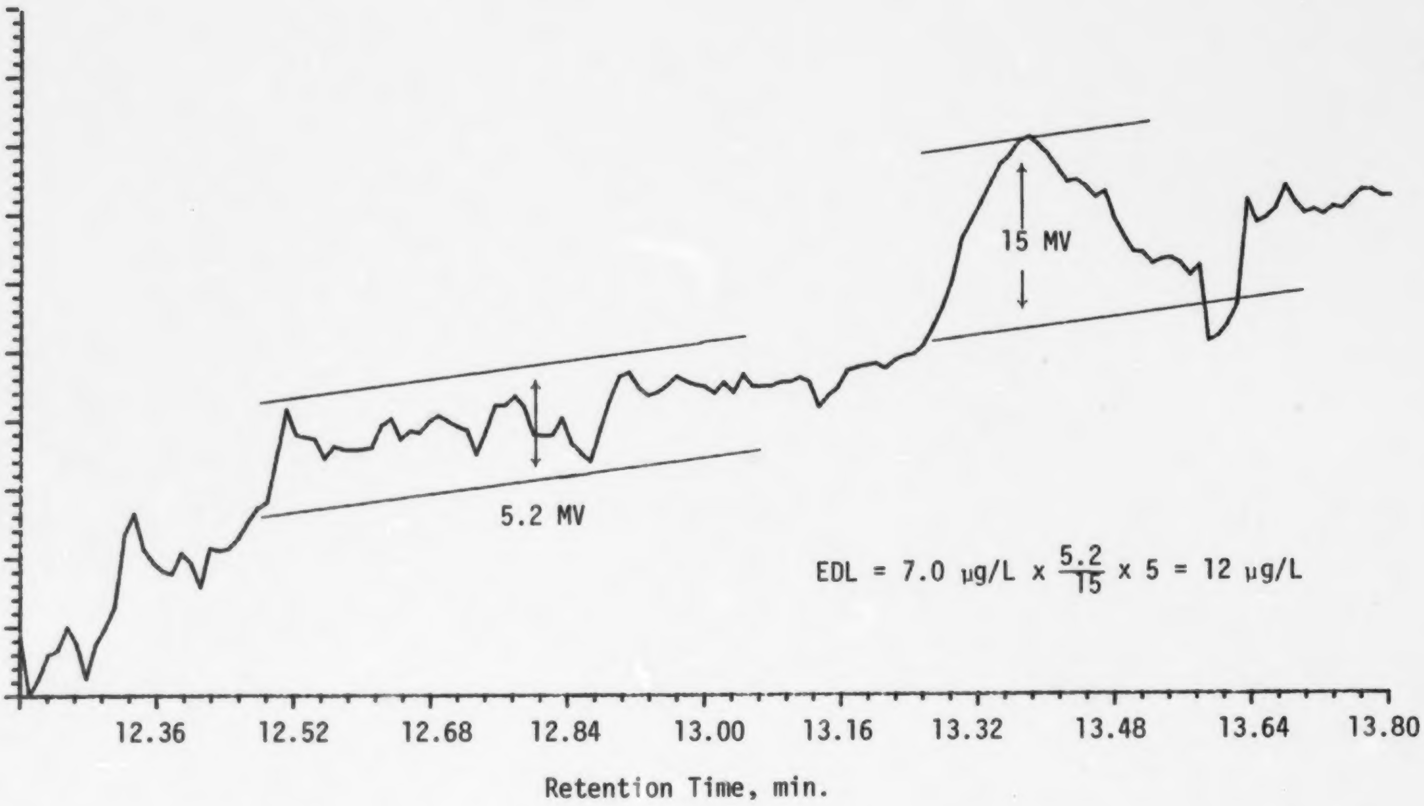


FIGURE 25. GC-NPD CHROMATOGRAM (USING BEAD 2) OF EPN STANDARD EQUIVALENT TO 7.0 μg/L IN WATER USED TO DETERMINE METHOD EDL.

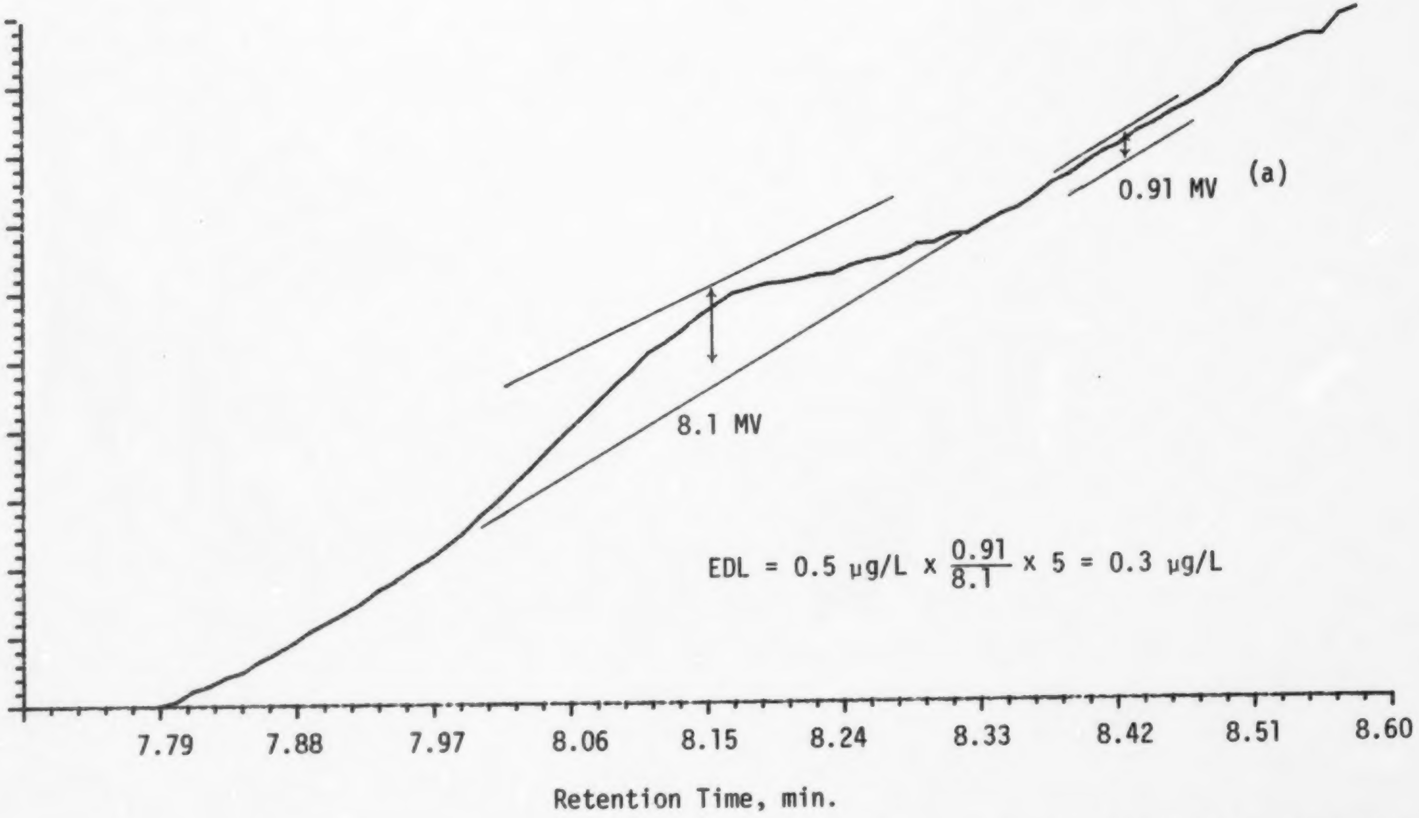


FIGURE 26. GC-NPD CHROMATOGRAM (USING BEAD 2) OF ETHION STANDARD EQUIVALENT TO 0.5 μg/L IN WATER USED TO DETERMINE METHOD EDL. (a) BASELINE DETERMINED FROM EXPANDED CHROMATOGRAM.

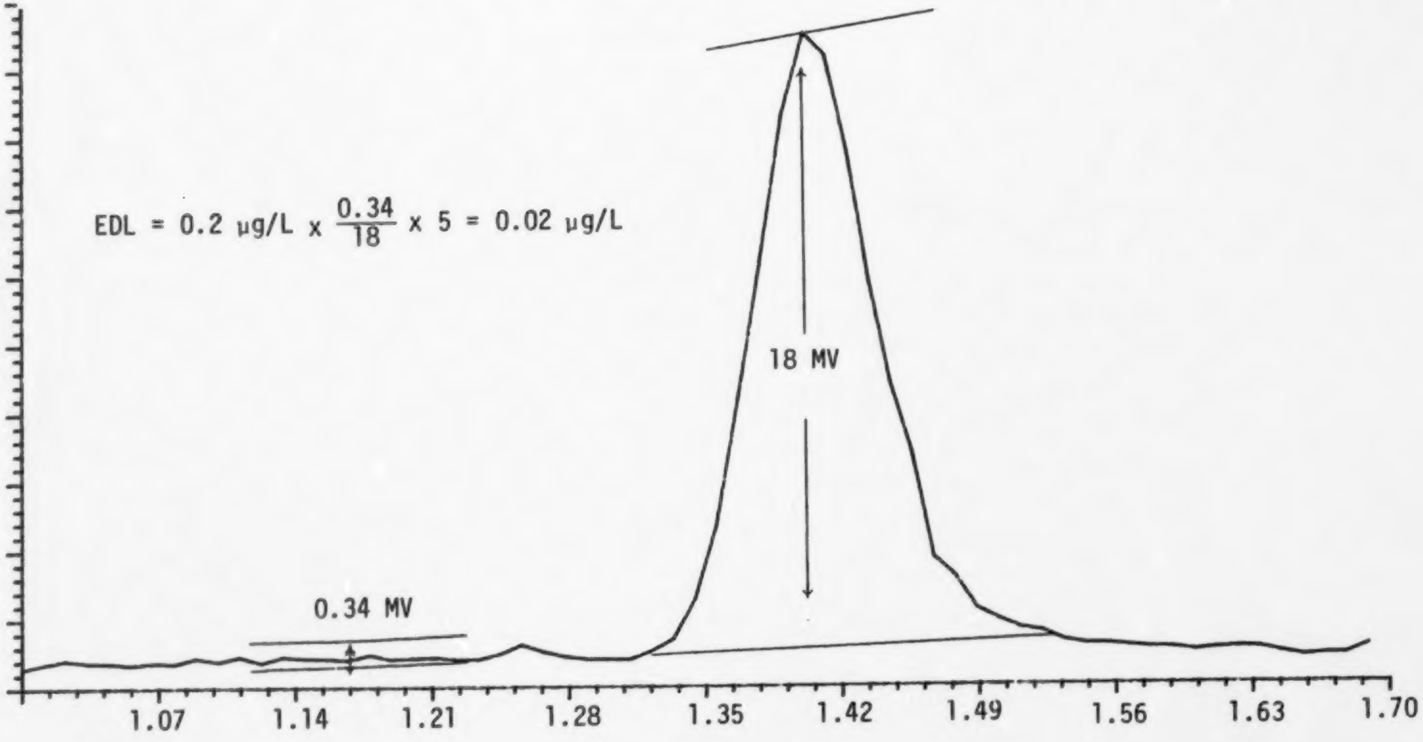


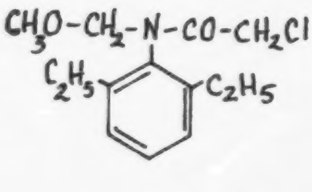
FIGURE 27. GC-NPD CHROMATOGRAM (USING BEAD 2) OF TERBUFOS STANDARD EQUIVALENT TO 0.2 µg/L IN WATER USED TO DETERMINE METHOD EDL.

SECTION 8

ANALYSIS OF CERTAIN AMINE PESTICIDES AND LETHANE IN WASTEWATER BY GAS CHROMATOGRAPHY

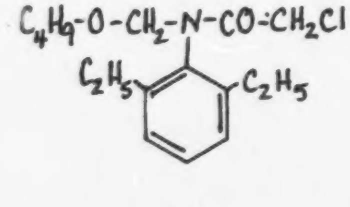
INTRODUCTION

Alachlor (XIV) is a selective preemergent herbicide. The IUPAC name for alachlor is 2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide and its CAS registry number is 15972-60-8. A common synonym for alachlor is Lasso. Alachlor is a crystalline solid with a melting point of 40-41°C. The solubility of alachlor in water is 240 mg/L at 23°C and it is soluble in most organic solvents. Alachlor is hydrolyzed under strongly alkaline or acidic conditions. The acute oral LD50 for rats for alachlor is 1200 mg/kg (67).



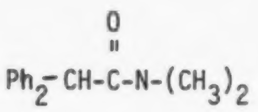
XIV

Butachlor (XV) is a preemergent herbicide. The IUPAC name for butachlor is N-(butoxymethyl)-2-chloro-2',6'-diethylacetanilide and its CAS registry number is 23184-66-9. A common synonym for butachlor is Machete. Butachlor is a light yellow oil. Butachlor has a solubility in water of 20 mg/L at 20°C and is soluble in most organic solvents. The acute oral LD50 for rats for butachlor is 3300 mg/kg (68).



XV

Diphenamid (XVI) is a preemergent herbicide. The IUPAC name for diphenamid is N,N-dimethyl-2,2-diphenylacetamide and its CAS registry number is 957-51-7. Common synonyms for diphenamid are Diphacin and Ramik. Diphenamid is a colorless crystalline solid with a melting point of 134.5 to 135.5°C. The solubility of diphenamid in water is 260 mg/L at 27°C and it is moderately soluble in most organic solvents. Diphenamid is stable in acids and alkali. The acute oral LD50 for rats for diphenamid is 1050 mg/kg (69).



$$\mathrm{Ph_2{-}CH{-}\overset{\overset{O}{\|}}{C}{-}N{-}(CH_3)_2}$$

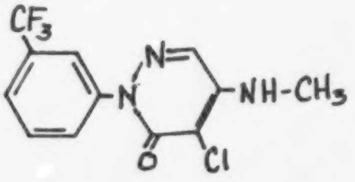
XVI

Lethane (XVII) is an insecticide. The CAS registry number for lethane is 112-56-1. A common synonym for lethane is Lethane 384. Lethane is practically insoluble in water. The acute oral LD50 for lethane for rats is 90 mg/kg.

$$\mathrm{C_4H_9{-}O{-}(CH_2)_2{-}O{-}(CH_2)_2{-}S{-}CN}$$

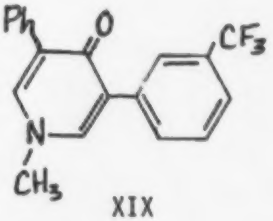
XVII

Norflurazon (XVIII) is a selective herbicide. The IUPAC name for norflurazon is 4-chloro-5-methylamino-2-(α,α,α-trifluoro-m-tolyl)pyridazin-3-one and its CAS registry number is 27314-13-2. Common synonyms for norflurazon are Zorial, Evital, and Solicam. Norflurazon is a colorless crystalline solid with a melting point of 174-180°C. The solubility of norflurazon in water is 28 mg/L at 23°C and it is soluble in most organic solvents. Norflurazon is stable under alkaline or acidic conditions, but is sensitive to light. The acute oral LD50 for rats for norflurazon is >8000 mg/kg (70).



XVIII

Fluridone (XIX) is a preemergent herbicide. The IUPAC name for fluridone is 1-methyl-3-phenyl-5-(α,α,α -trifluoro-m-tolyl)-4-pyridone and its CAS registry number is 59756-60-4. Fluridone is an off-white crystalline solid with a melting point of 151-154°C. Fluridone has a solubility in water of 12 mg/L at pH 7 and is soluble in polar organic solvents. Fluridone is stable to hydrolysis at pH 3-9 but is moderately susceptible to decomposition by light. The acute oral LD50 for rats for fluridone is >10,000 mg/kg (71).



XIX

Analysis methods used to determine the amine pesticides and lethane in various matrices have been reported in the literature. These methods usually use GC coupled with an NPD or ECD (24,59,72,73,74,75,77,79,80). Several methods use HPLC with a UV detector (76,78,81,82).

The method provided by the Project Officer for evaluation for determination of amine pesticides and lethane consisted of adjustment of the sample pH to the range of 5 to 9 by addition of dilute sodium hydroxide or sulfuric acid; extraction of the sample with methylene chloride using a separatory funnel; drying of the sample using anhydrous sodium sulfate; concentration of the sample to 10 mL after exchanging the solvent with hexane; and analysis of the sample extract by packed column GC-NPD. A cleanup procedure using 2 percent deactivated Florisil was included in the method.

CONCLUSIONS

A method designed to determine amine pesticides and lethane in wastewaters was evaluated. The extraction, cleanup and analysis portions of the method were found to be acceptable for the determinations of alachlor, butachlor, diphenamid, lethane, norflurazon, and fluridone in reagent water and POTW effluent; recoveries of these compounds spiked into these matrices at the 10 and 100 µg/L levels was generally greater than 80 percent both before and after the cleanup step.

EXPERIMENTAL

The following procedure was outlined in the amine pesticide and lethane analysis method:

1. Place sample (approximately 1 liter) into a 2-liter separatory funnel, and adjust the pH of the sample to

within the range of 5 to 9 with dilute sodium hydroxide or sulfuric acid.

2. Extract the sample with three 60-mL portions of methylene chloride and combine the extracts.

3. Dry the combined extracts by pouring through a chromatography column containing 10 cm of anhydrous sodium sulfate.

4. Concentrate the combined extracts to 1 mL using K-D equipment with the water bath at 80 to 85°C. Increase the temperature of the water bath to 90-95°C. Add 50 mL of hexane to the sample and reconcentrate the sample to a final volume of 10 mL.

5. A cleanup procedure using 2 percent deactivated Florisil is used for samples that require cleanup. Florisil, 20 grams, is slurried in hexane and placed in a chromatography column. The sample is applied to the column in hexane, and the column is eluted with 100 mL of hexane which is discarded. The column is then eluted with 200 mL of 6 percent acetone in hexane (F1) and 200 mL of 15 percent acetone in hexane (F2). Alachlor, butachlor, and lethane elute in F1. Diphenamid elutes in F2. A cleanup procedure for norflurazon and fluridone was not included. Each fraction is concentrated to 10 mL using K-D equipment with the water bath at 90 to 95°C. A hexane solvent exchange step is included.

6. Analyze the sample for alachlor, butachlor, diphenamid, lethane, and norflurazon by GC-NPD using the following conditions:
   - 180 cm long by 2 mm ID glass column packed with 10 percent OV-11 on 100/120 mesh Gas-Chrom WHP;
   - nitrogen carrier gas at 30 mL/min flow rate;
   - oven temperature of 225°C isothermal for 4 min, programmed to 275°C at 4°C/min, held at 275°C for 8 minutes;
   - injection volume of 5 µL.

7. Analyze the sample for fluridone by GC-NPD using the following conditions:
   - 180 cm long by 2 mm ID glass column packed with 3 percent Dexsil 300 on 80/100 mesh Gas-Chrom WHP;
   - nitrogen carrier gas at 30 mL/min flow rate;
   - oven temperature of 300°C isothermal;
   - injection volume of 5 µL.

Samples analyzed included the following:

1. Triplicate reagent water samples.

2. Triplicate reagent water samples spiked with the amine pesticides and lethane at the 10 μg/L concentration level.

3. Triplicate reagent water samples spiked with the amine pesticides and lethane at the 100 μg/L concentration level.

4. Triplicate POTW secondary effluent samples. POTW secondary effluent was obtained from the City of Columbus.

5. Triplicate POTW secondary effluent samples spiked with the amine pesticides and lethane at the 10 μg/L concentration level.

6. Triplicate POTW secondary effluent samples spiked with the amine pesticides and lethane at the 100 μg/L concentration level.

All extracts described in points 1-6 were analyzed and then treated to the Florisil cleanup procedure and reanalyzed by GC-NPD.

Recoveries were determined by comparison to standard solutions of the amine pesticides and lethane prepared at the 0.5, 1.0, and 10 μg/mL concentration levels in hexane. These concentration levels were equivalent to 5.0, 10, and 100 μg/L of the amine pesticides and lethane in the original samples assuming a final extract volume of 10 mL. Response factors were calculated for the standards and used to determine concentration levels in the abovementioned reagent water and POTW effluent samples.

The method EDLs, defined as the concentration of each amine pesticide or lethane in a sample yielding a S/N of 5, were determined by injecting 0.05 μg/mL solutions of alachlor, butachlor, and diphenamid, 0.01 μg/mL solutions of lethane and norflurazon, and a 0.1 μg/mL solution of fluridone in hexane.

QUALITY ASSURANCE

Instrumentation was set up as described in the method. Calibration standards used to analyze the samples before cleanup were prepared containing the amine pesticides and lethane at the 0.5, 1.0, and 10 μg/mL concentration levels in hexane. These are equivalent to compound concentrations of 5.0, 10, and 100 μg/L in a water sample. These calibration standards were analyzed in duplicate prior to analyses of the water extracts; a calibration standard was analyzed after every five water extracts. Response factors were calculated for each calibration run by dividing the concentration level in μg/L by the corresponding peak area. Resultant calibration data are reported in Table 18. Response factors were repeatable over the entire calibration range. Response factors of 0.019, 0.023, 0.012, 0.013, 0.0078, and 0.021 were used for alachlor, butachlor, diphenamid, lethane, norflurazon, and fluridone concentration determinations, respectively.

TABLE 18. AMINE PESTICIDES AND LETHANE CALIBRATION DATA

Compound	Concentration, ug/L(a)	Average Peak Area	Standard Deviation, %	Number of Replicates	Average Response Factors
Alachlor	0.5	26	3	4	0.019
Alachlor	1.0	53	3	4	0.019
Alachlor	10	550	2	4	0.018
Butachlor	0.5	21	2	4	0.023
Butachlor	1.0	44	3	4	0.023
Butachlor	10	443	2	4	0.023
Diphenamid	0.5	42	2	4	0.012
Diphenamid	1.0	85	3	4	0.011
Diphenamid	10	885	2	4	0.011
Lethane	0.5	31	9	4	0.016
Lethane	1.0	65	7	4	0.015
Lethane	10	797	6	4	0.013
Norflurazon	0.5	68	6	5	0.0073
Norflurazon	1.0	150	7	4	0.0067
Norflurazon	10	1560	2	4	0.0064
Fluridone	0.5	26	15	4	0.019
Fluridone	1.0	45	4	4	0.022
Fluridone	10	496	0	4	0.020

(a) Equivalent concentration of compound in water sample.

Standards of alachlor, butachlor, diphenamid, lethane, and norflurazon were obtained from two independent sources. Calibration standards used for quantification of these compounds in the water samples were prepared from compounds obtained from the reference standards repository of EPA-HERL. A test standard was prepared containing each of the compounds at the 10 μg/mL concentration level in hexane using compounds obtained from a chemical supply house. This test calibration standard was analyzed in triplicate and the resultant peak areas were compared to those generated from analyses of the original calibration standards. Resultant data indicated that for alachlor, diphenamid, lethane, and norflurazon the standard obtained from the chemical supply house was as pure as that obtained from the reference standards repository. Peak areas obtained from the test calibration standards of these compounds did not vary by more than five percent from peak areas obtained from the original calibration standards of these compounds. The standard of butachlor obtained from the chemical supply house was not as pure as that obtained from the reference standards repository. Peak areas obtained from the test calibration standard of butachlor were 26 percent lower than those obtained from the original calibration standard.

RESULTS AND DISCUSSION

Very low levels of norflurazon or a compound coeluting with norflurazon were detected in unspiked POTW effluent. This interference did not hinder determination of any of the amine pesticides or lethane. Chromatograms obtained during the analyses of reagent water and POTW effluent, unspiked and spiked with the amine pesticides and lethane at the 10 µg/L level are shown in Figures 28-31. The silica gel cleanup step was not applied to these samples.

Recoveries of the amine pesticides and lethane from water were generally greater than 80 percent, before and after cleanup. This indicates that the extraction, analysis, and cleanup procedures specified were effective. Although a cleanup procedure was not specified for norflurazon, recovery data was obtained for this compound after silica gel cleanup. All recovery data are given in Table 19.

The sensitivity of the method, as measured by the EDLs, was determined to be approximately 10 to 100 times higher than specified in the method. The EDLs for alachlor, butachlor, diphenamid, lethane, norflurazon, and fluridone were 0.2, 0.3, 0.2, 0.1, 0.02, and 0.6 µg/L, respectively. The chromatograms used to calculate these EDLs are shown in Figures 32-36.

RECOMMENDATIONS

The extraction, analysis, and cleanup procedures for the determination of the amine pesticides and lethane are acceptable.

TABLE 19. RECOVERIES OF AMINE PESTICIDES AND LETHANE FROM WATER[c]

Compound	Spike Level, µg/L	Matrix[a]	Before Cleanup			After Cleanup		
			Blank Level	Recovery, %	Standard Deviation,%	Blank Level	Recovery, %	Standard Deviation,%
Alachlor	10	1	ND[b]	96	3	ND	96	3
Alachlor	100	1	ND	94	2	ND	94	3
Alachlor	10	2	ND	109	1	ND	105	3
Alachlor	100	2	ND	102	1	ND	97	3
Butachlor	10	1	ND	96	4	ND	95	3
Butachlor	100	1	ND	93	1	ND	93	2
Butachlor	10	2	ND	103	1	ND	104	5
Butachlor	100	2	ND	100	0	ND	95	2
Diphenamid	10	1	ND	93	6	ND	95	2
Diphenamid	100	1	ND	94	2	ND	97	3
Diphenamid	10	2	ND	105	1	ND	95	3
Diphenamid	100	2	ND	103	1	ND	94	4
Lethane	10	1	ND	93	6	ND	97	2
Lethane	100	1	ND	100	1	ND	99	4
Lethane	10	2	ND	119	2	ND	108	4
Lethane	100	2	ND	123	1	ND	106	3
Norflurazon	10	1	ND	69	10	ND	69	6
Norflurazon	100	1	ND	92	1	ND	60	6
Norflurazon	10	2	0.02	107	1	ND	76	11
Norflurazon	100	2	0.02	108	2	ND	65	17
Fluridone	10	1	ND	49	16	--	--	--
Fluridone	100	1	ND	81	15	--	--	--
Fluridone	10	2	ND	124	6	--	--	--
Fluridone	100	2	ND	111	2	--	--	--

(a) 1=Reagent water; 2=Columbus POTW secondary effluent.
(b) ND=not detected.
(c) Recovery data are averages of three replicate analyses.

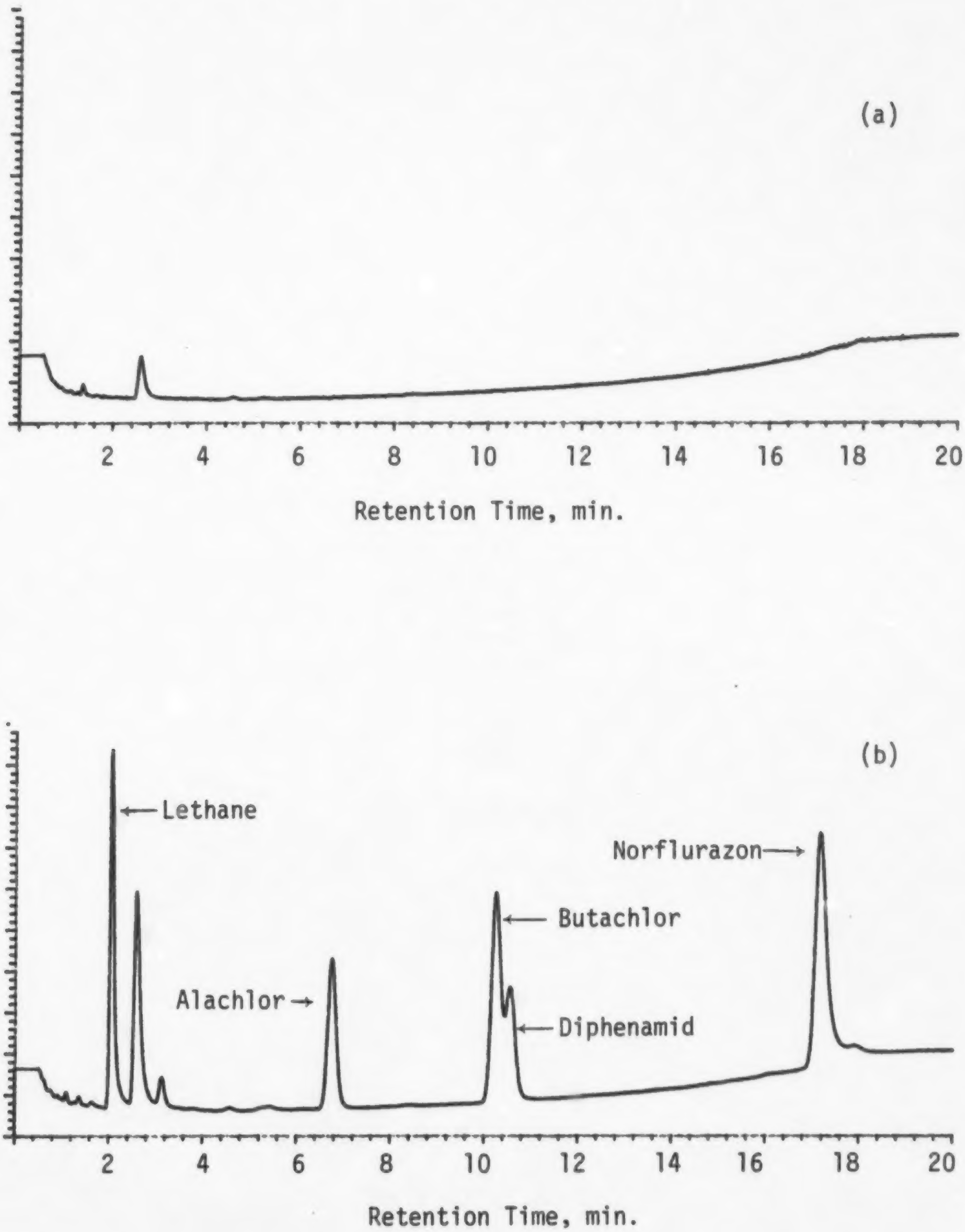


FIGURE 28. GC-NPD CHROMATOGRAM OF (a) REAGENT WATER, AND (b) REAGENT WATER SPIKED WITH ALACHLOR, BUTACHLOR, DIPHENAMID, LETHANE, AND NORFLURAZON AT THE 10 μg/L LEVEL.

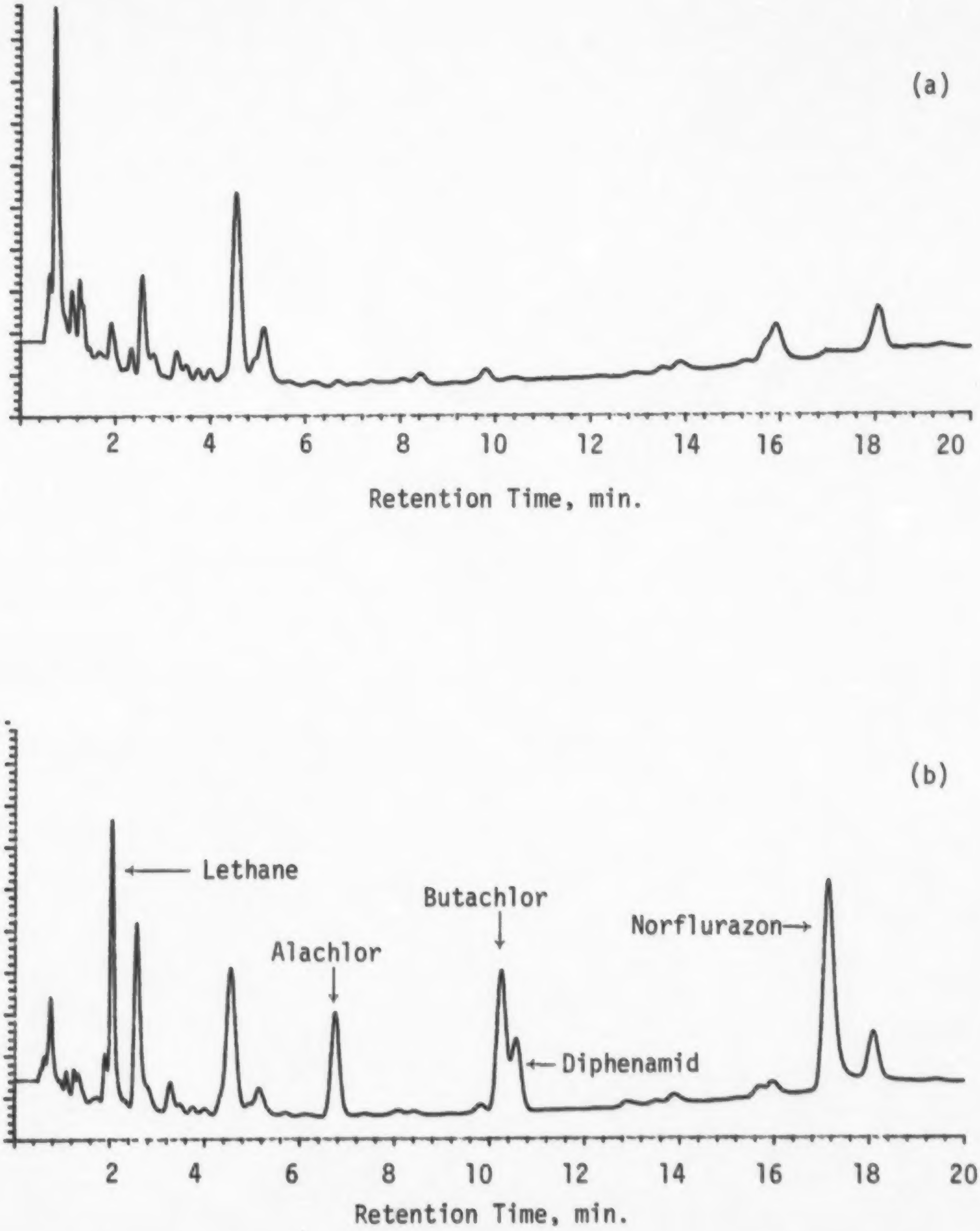


FIGURE 29. GC-NPD CHROMATOGRAM OF (a) POTW EFFLUENT, AND (b) POTW EFFLUENT SPIKED WITH ALACHLOR, BUTACHLOR, DIPHENAMID, LETHANE AND NORFLURAZON AT THE 10 μg/L LEVEL.

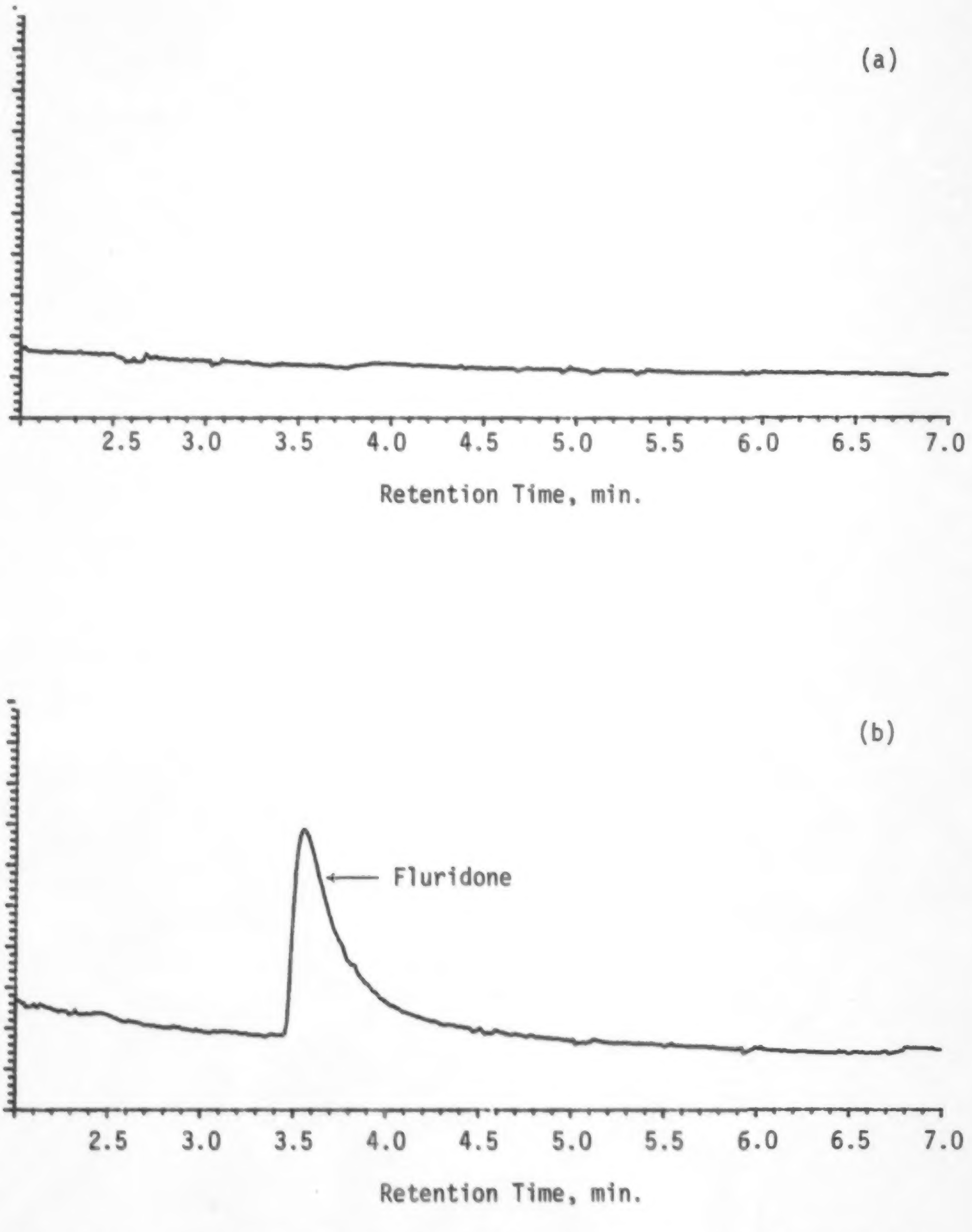


FIGURE 30. GC-NPD CHROMATOGRAM OF (a) REAGENT WATER, AND (b) REAGENT WATER SPIKED FLURIDONE AT THE 10 µg/L LEVEL.

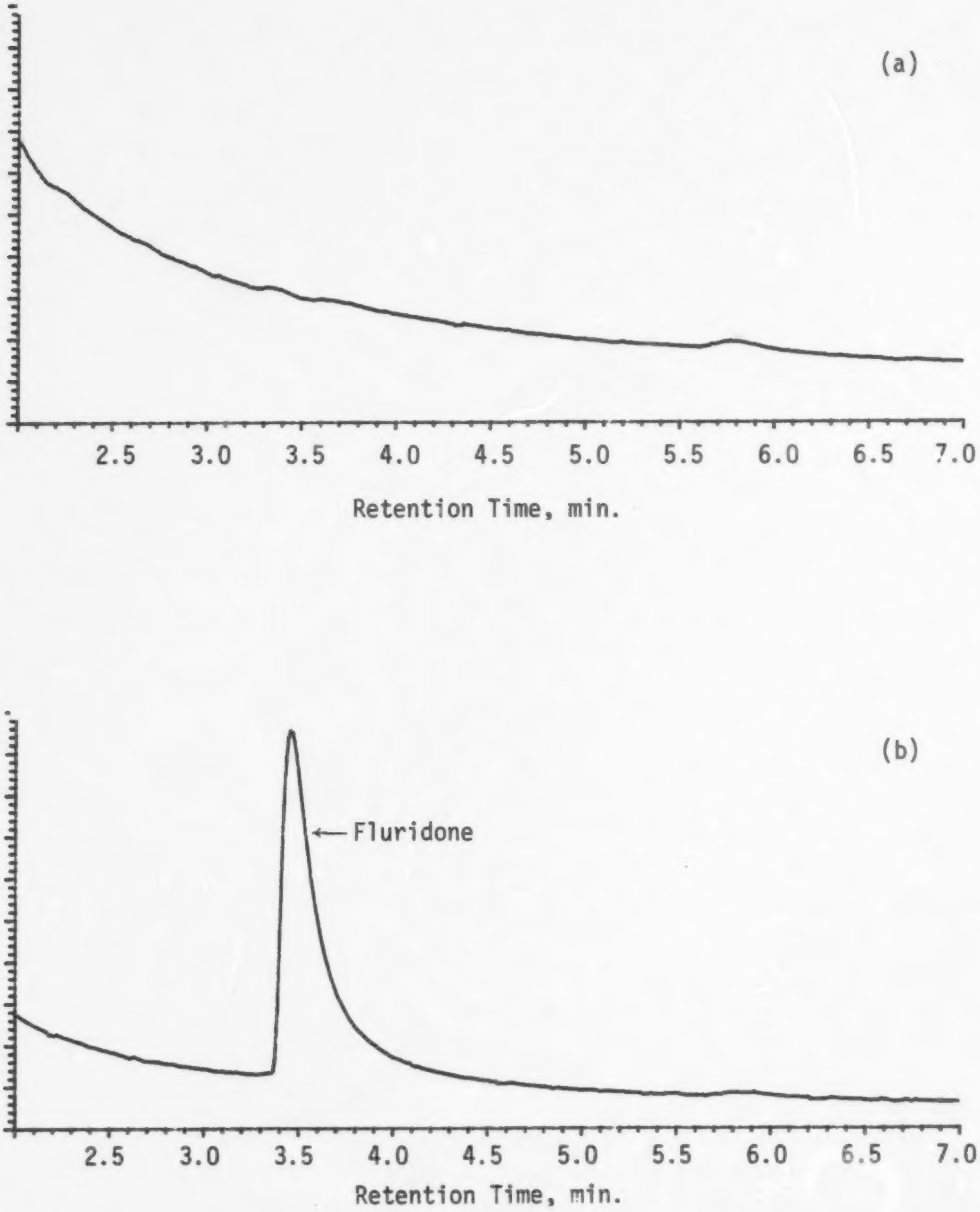
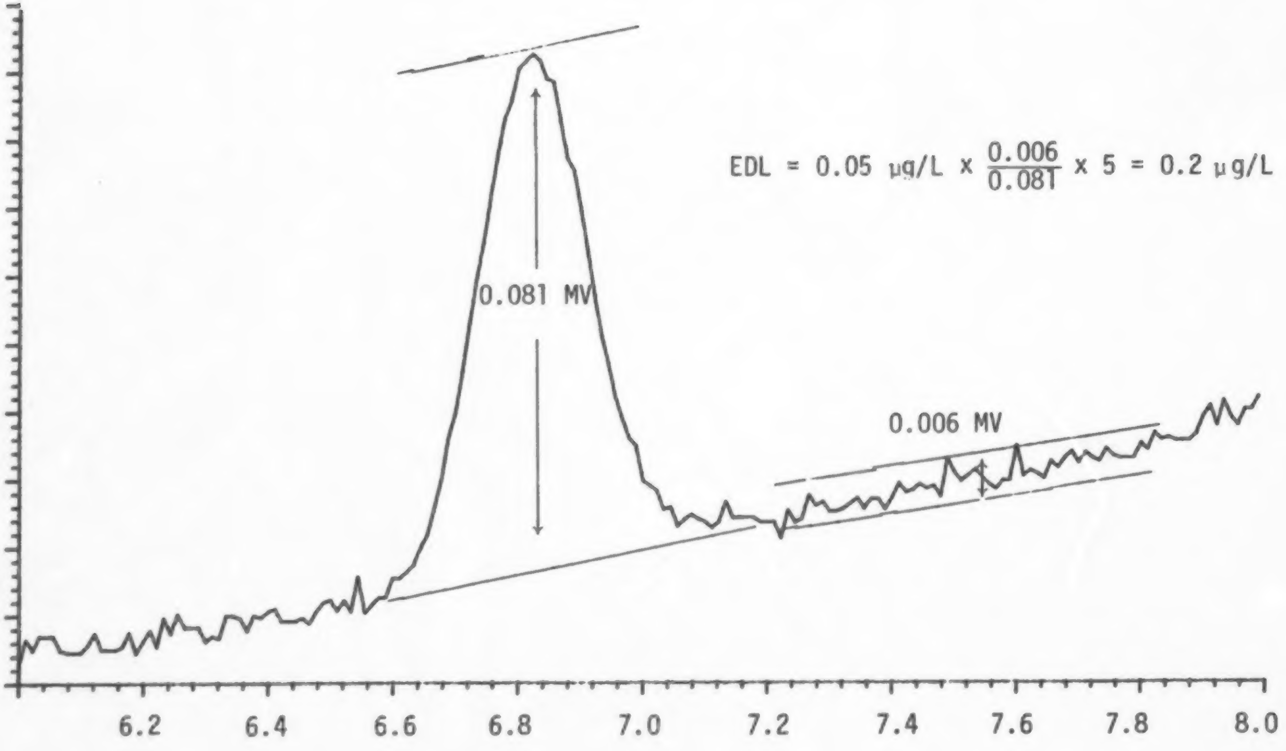


FIGURE 31. GC-NPD CHROMATOGRAM OF (a) POTW EFFLUENT, AND (b) POTW EFFLUENT SPIKED WITH FLURIDONE AT THE 10 μg/L LEVEL.



$$EDL = 0.05\ \mu g/L \times \frac{0.006}{0.081} \times 5 = 0.2\ \mu g/L$$

FIGURE 32. GC-NPD CHROMATOGRAM OF ALACHLOR SOLUTION REPRESENTING 0.5 µg/L IN WATER USED TO DETERMINE METHOD EDL.

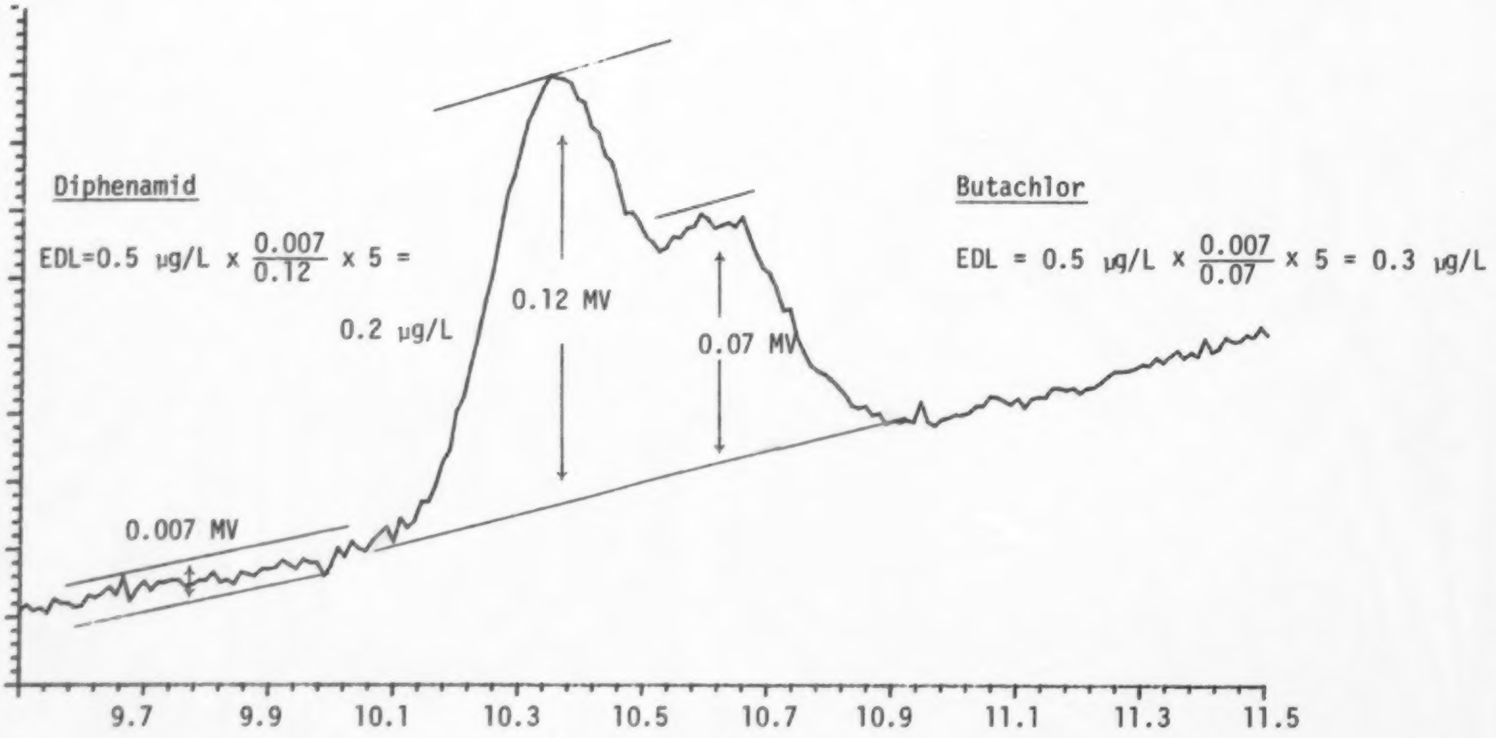


FIGURE 33. GC-NPD CHROMATOGRAM OF BUTACHLOR AND DIPHENAMID SOLUTION REPRESENTING 0.5 µg/L OF EACH IN WATER USED TO DETERMINE METHOD EDL.

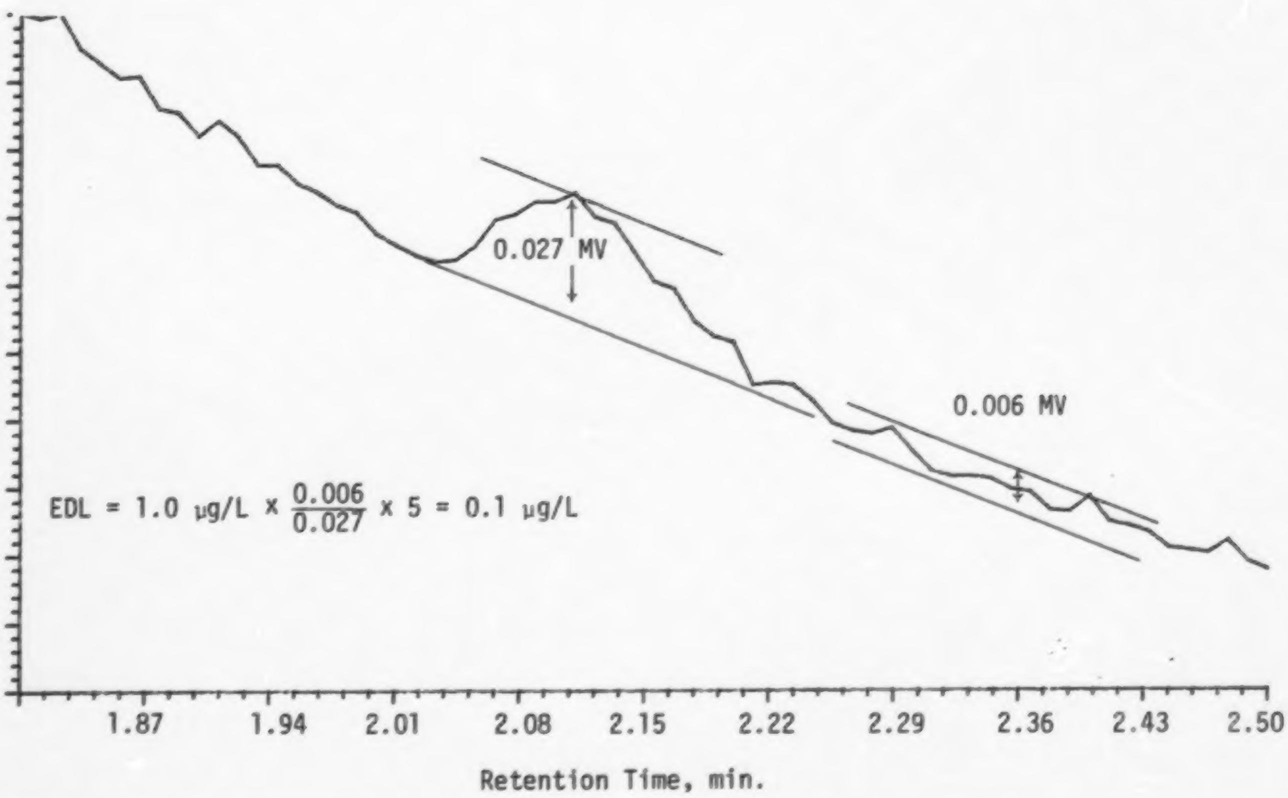


FIGURE 34. GC-NPD CHROMATOGRAM OF LETHANE SOLUTION REPRESENTING 1.0 μg/L IN WATER USED TO DETERMINE THE METHOD EDL.

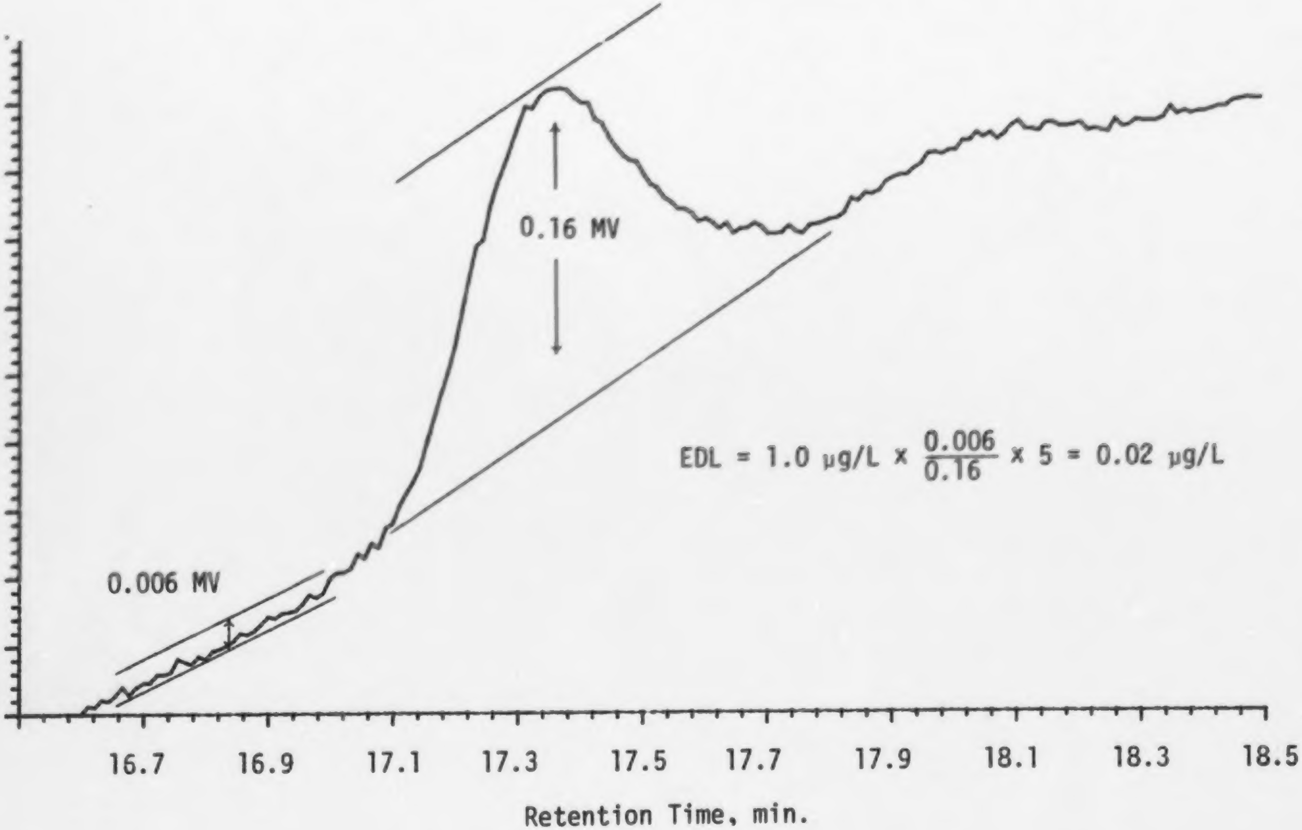
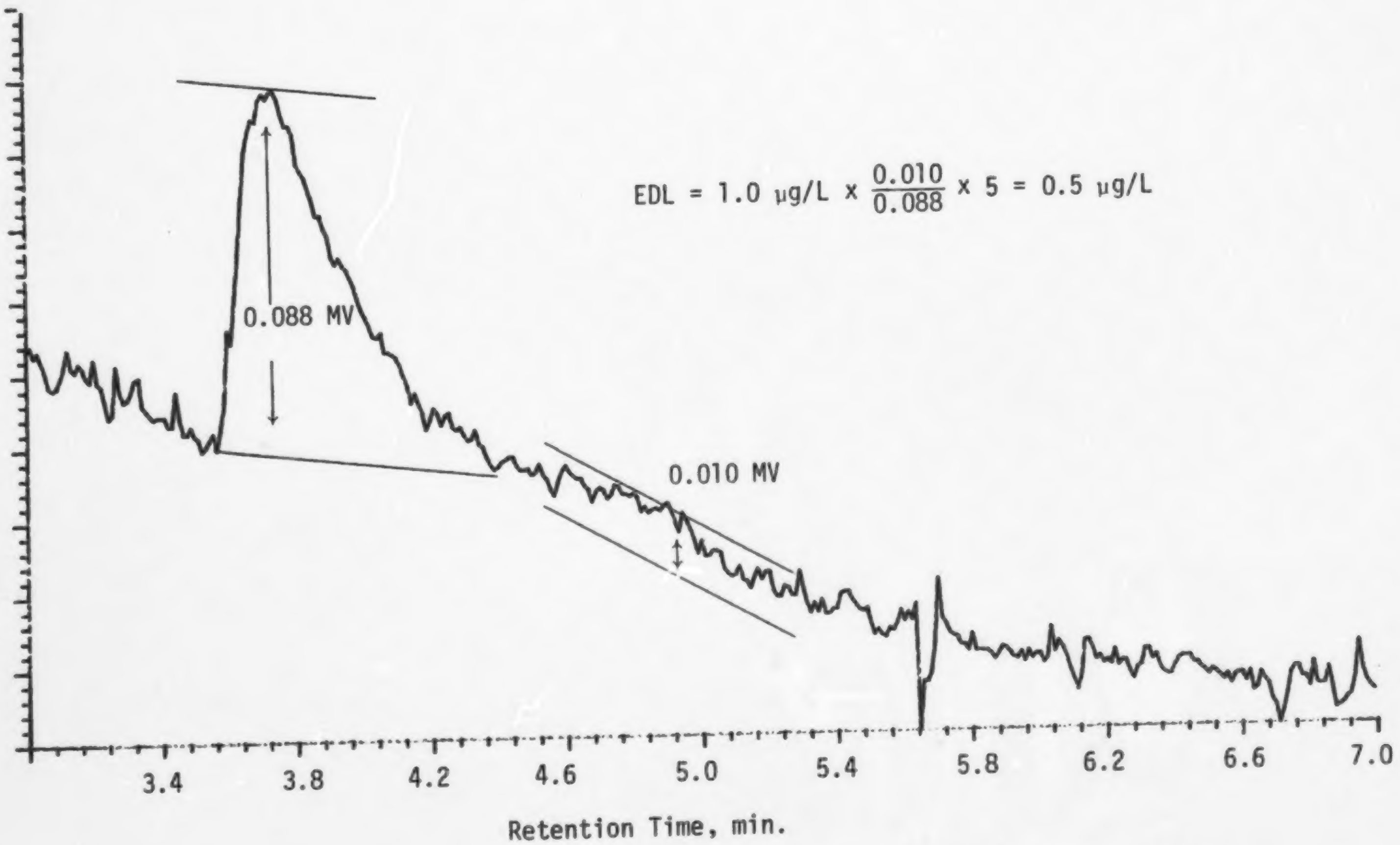


FIGURE 35. GC-NPD CHROMATOGRAM OF NORFLURAZON SOLUTION REPRESENTING 1.0 μg/L IN WATER USED TO DETERMINE METHOD EDL.



$$\text{EDL} = 1.0\ \mu\text{g/L} \times \frac{0.010}{0.088} \times 5 = 0.5\ \mu\text{g/L}$$

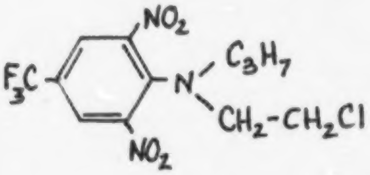
FIGURE 36. GC-NPD CHROMATOGRAM OF FLURIDONE SOLUTION REPRESENTING 1.0 µg/L IN WATER USED TO DETERMINE METHOD EDL.

SECTION 9

ANALYSIS OF DINITRO AROMATIC PESTICIDES IN WASTEWATER BY GAS CHROMATOGRAPHY

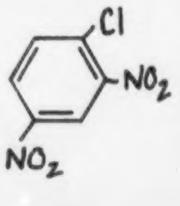
INTRODUCTION

Basalin (XX) is a preemergent herbicide. The IUPAC name for basalin is N-2-chloroethyl-α,α,α-trifluoro-2,6-dinitro-N-propyl-p-toluidine and its CAS registry number is 33245-39-5. A common synonym for basalin is fluchoralin. Basalin forms orange-yellow crystals with a melting point of 42-43°C. The solubility of basalin in water is 70 mg/L at 20°C. The acute oral LD50 for basalin for rats is 1110 mg/kg.



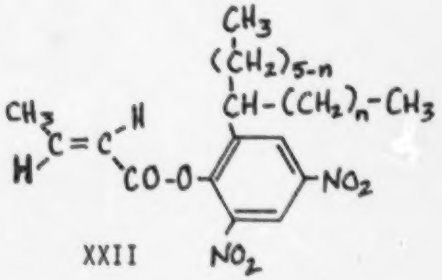
XX

CDNB (XXI) has an IUPAC name of 1-chloro-2,4-dinitrobenzene and its CAS registry number is 97-00-7. CDNB forms yellow crystals with a melting point of 52-54°C. CDNB is practically insoluble in water and soluble in most organic solvents. The acute oral LD50 for rats for CDNB is 1076 mg/kg.

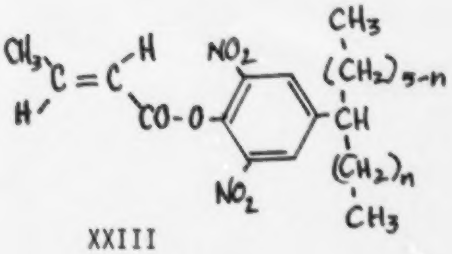


XXI

Dinocap is a nonsystemic acaracide and contact fungicide. Dinocap is a mixture of 2,4-dinitro-6-octylphenyl crotonates (XXII) and 2,6-dinitro-4-octylphenyl crotonates (XXIII) and its CAS registry number is 34300-45-3. Dinocap is a dark brown liquid which is insoluble in water and soluble in most organic solvents. The acute oral LD50 for rats for dinocap is 980-1190 mg/kg (83).



XXII



XXIII

Several analyses methods for the determination of the dinitro aromatic pesticides in various matrices have been reported. A method was reported for the determination of dinocap in produce by GC-ECD (35). Methods were also reported to determine basalin in formulations by GC-FID (84) and to determine CDNB by HPLC (85).

The method provided by the Project Officer for evaluation for determination of dinitro aromatic pesticides consisted of adjustment of the sample pH to the range of 5 to 9 by addition of dilute sodium hydroxide or sulfuric acid; extraction of the sample with 15 percent methylene chloride in hexane using a separatory funnel; drying of the sample using anhydrous sodium sulfate; concentration of the sample to 1 mL after exchanging the solvent with hexane; and analysis of the sample extract by packed column GC-ECD. A cleanup procedure using Florisil was included in the method.

CONCLUSIONS

A method designed to determine dinitro aromatic pesticides in wastewaters was evaluated. The extraction, cleanup, and analysis portions of the method were found to be acceptable for determination of basalin in reagent water and POTW effluent and marginally acceptable for the determination of CDNB and dinocap in reagent water and POTW effluent. Recoveries of basalin spiked into these matrices at the 0.1 and 1.0 µg/L levels were generally greater than 90 percent both before and after the cleanup step. Recoveries of CDNB and dinocap spiked into these matrices at the 0.1 and 1.0 µg/L levels were generally greater than 70 percent both before and after the cleanup step.

EXPERIMENTAL

The following procedure was outlined in the dinitro aromatic pesticide analysis method:

1. Place sample (approximately 1 liter) into a 2-liter separatory funnel, and adjust the pH of the sample to within the range of 5 to 9 with dilute sodium hydroxide or sulfuric acid.

2. Extract the sample with three 60-mL portions of 15 percent methylene chloride in hexane and combine the extracts.

3. Dry the combined extracts by pouring through a chromatography column containing 10 cm of anhydrous sodium sulfate.

4. Concentrate the combined extracts to 1 mL using K-D equipment with the water bath at 90 to 95°C. A hexane solvent exchange step is included.

5. A Florisil cleanup procedure is used for samples that require cleanup. A specified amount of Florisil, determined by its lauric acid value, is placed in a chromatography column. The sample is applied to the column in hexane, and the column is eluted with 30 mL of 50 percent methylene chloride in hexane, which is discarded. The column is then eluted with 30 mL of methylene chloride (F1) and 30 mL of 10 percent acetone in methylene chloride (F2). Basalin and CDNB elute in F1, and dinocap elutes in F2. Each fraction is concentrated to 1 mL using K-D equipment with the water bath at 80 to 85°C. A hexane solvent exchange step is included.

6. Analyze the sample by GC-ECD using the following conditions:
   - 180 cm long by 2 mm ID glass column packed with 1.5 percent OV-17/1.95 percent OV-210 on Supelcoport (100/120 mesh);

- 5 percent methane/95 percent argon carrier gas at 33 mL/min flow rate;
- oven temperature of 160°C isothermal for basalin and CDNB and 200°C isothermal for dinocap;
- injection volume of 5 μL.

These conditions were evaluated prior to analysis of water extracts, and it was found that the dinitro aromatic pesticides eluted from the GC column much later than specified in the analysis method. The method indicates that basalin and CDNB should elute at 6.4 and 2.0 minutes, respectively, and that dinocap should elute from 10 to 16 minutes. Instead, dinocap eluted from 36 to 51 minutes, and basalin and CDNB did not elute from the column. The oven temperatures used for GC analysis of the samples were raised to 200°C for basalin and CDNB, and to 230°C for dinocap. With the increased oven temperatures, basalin elutes at 4.8 minutes, CDNB elutes at 3.0 minutes, and dinocap elutes between 11 and 15 minutes. Chromatograms showing the elution profiles of basalin and CDNB at 170°C and 200°C are given in Figure 37. Chromatograms showing the elution profiles of dinocap at 200°C and 230°C are given in Figure 38.

The EDLs for the dinitro aromatic pesticides originally listed in the method were 1.0 μg/L for all three compounds. This was estimated to be too high; EDLs of 0.01 μg/L were considered to be more likely. After consultation with the EPA Task Officer, it was agreed that method evaluations would be done using compound spike levels of 0.1 and 1.0 μg/L.

Samples processed included the following:

1. Triplicate reagent water samples. Reagent water was obtained from a Millipore system.

2. Triplicate reagent water samples spiked with the dinitro aromatic pesticides at the 0.1 μg/L concentration level.

3. Triplicate reagent water samples spiked with the dinitro aromatic pesticides at the 1.0 μg/L concentration level.

4. Triplicate POTW secondary effluent samples. POTW secondary effluent was obtained from the City of Columbus.

5. Triplicate POTW secondary effluent samples spiked with the dinitro aromatic pesticides at the 0.1 μg/L concentration level.

6. Triplicate POTW secondary effluent samples spiked with the dinitro aromatic pesticides at the 1.0 μg/L concentration level.

All extracts described in points 1-6 were analyzed and then treated to Florisil cleanup procedure and reanalyzed by GC-ECD.

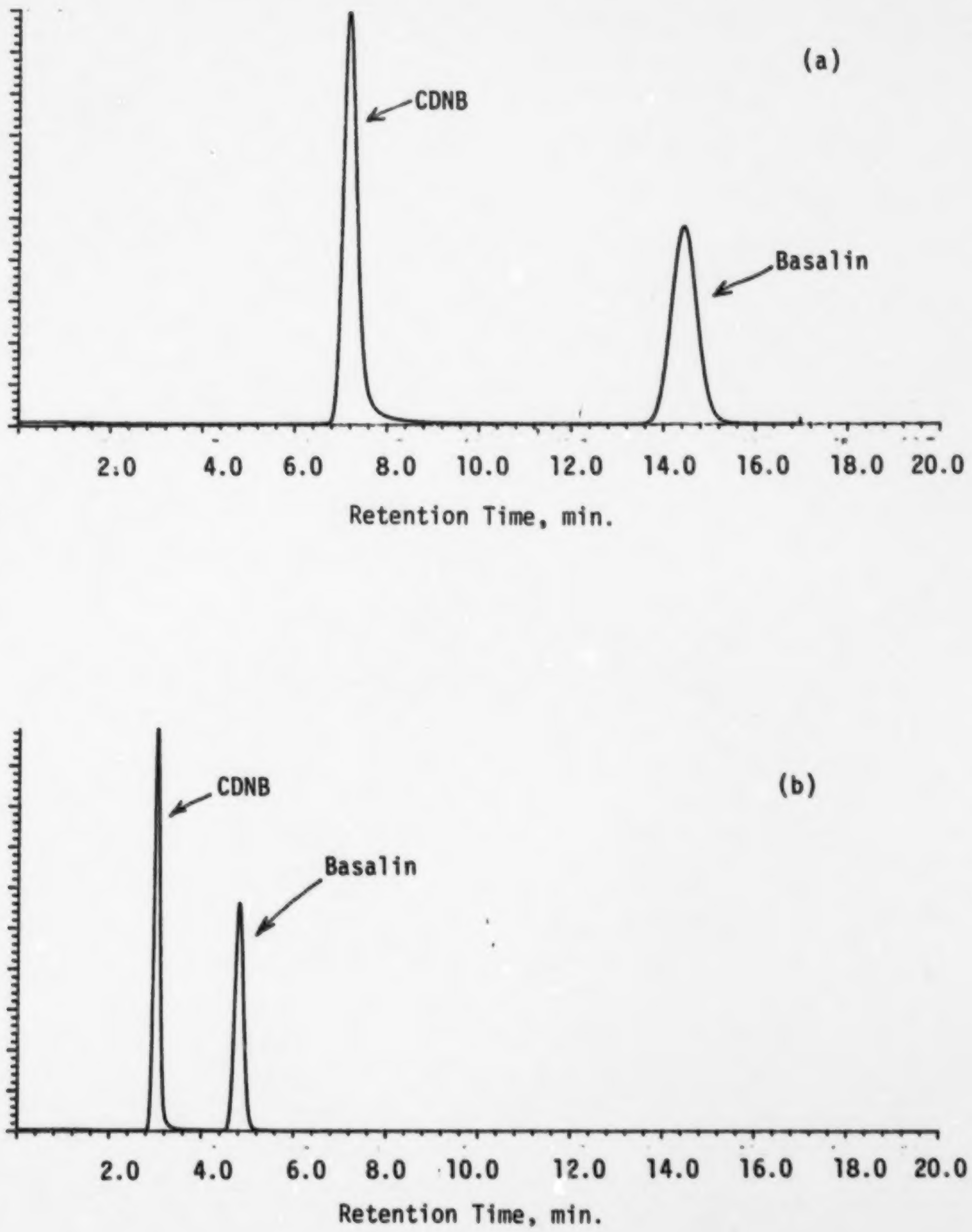


FIGURE 37. GC-ECD CHROMATOGRAMS OF HEXANE SOLUTION CONTAINING BASALIN AND CDNB USING OVEN TEMPERATURES OF (a) 170°C and (b) 200°C.

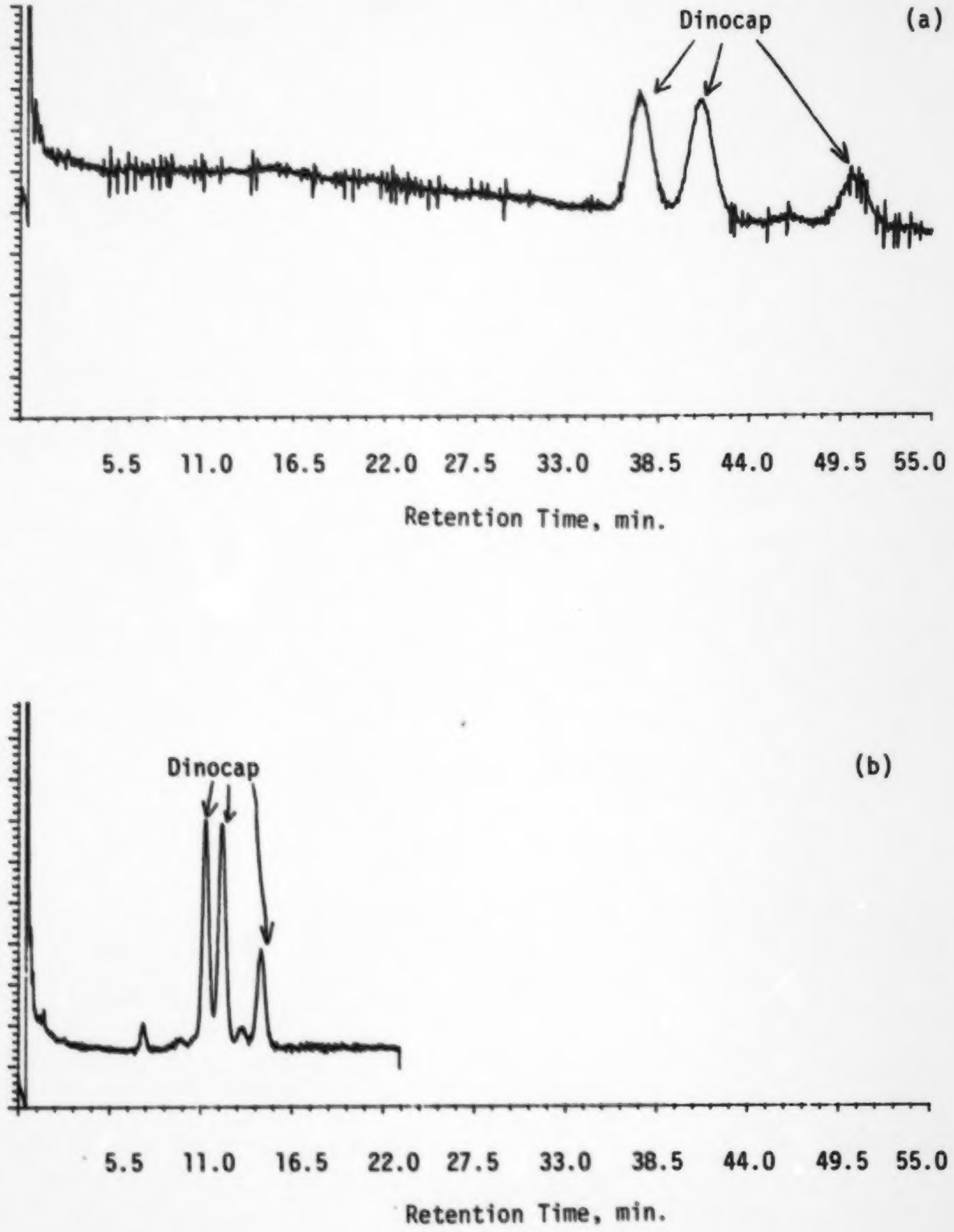


FIGURE 38. GC-ECD CHROMATOGRAMS OF HEXANE SOLUTION CONTAINING DINOCAP USING OVEN TEMPERATURES OF (a) 200°C and (b) 230°C.

Recoveries were determined by comparison to standard solutions of basalin and CDNB prepared at the 0.05, 0.1, and 1.0 µg/mL concentration levels and dinocap prepared at the 0.1, 1.0 and 1.5 µg/mL levels in hexane. These concentration levels were equivalent to 0.05, 0.1, and 1.0 µg/L of basalin and CDNB and 0.1, 1.0, and 1.5 µg/L of dinocap in the original samples assuming a final extract volume of 1 mL. Response factors were calculated for the standards and used to determine concentration levels in the abovementioned reagent water and POTW effluent samples.

The method EDLs, defined as the concentration of each dinitro aromatic pesticide in a sample yielding a S/N of 5, were determined by injecting 0.0005 µg/mL standard solutions of basalin and CDNB and a 0.05 µg/mL standard solution of dinocap prepared in hexane. The solutions were equivalent to basalin and CDNB concentrations of 0.0005 µg/L and a dinocap concentration of 0.05 µg/L in a water sample.

QUALITY ASSURANCE

Instrumentation was set up as described in the method. Sensitivity achieved was approximately three orders of magnitude lower than that reported in the method for basalin and CDNB and one order of magnitude lower than that reported for dinocap. Calibration standards consisted of basalin and CDNB solutions prepared at the 0.05, 0.1, and 1.0 µg/mL concentration levels and dinocap solutions prepared at the 0.1,, 1.0, and 1.5 µg/mL concentration levels in hexane. Each calibration standard was analyzed in duplicate prior to analysis of any evaluation samples. A selected calibration standard was then analyzed after every five evaluation samples. Response factors were calculated for each calibration run by dividing the equivalent pesticide concentration level in the sample in µg/L by the pesticide peak area. Resultant calibration data are reported in Table 20. Response factors were repeatable over the entire calibration range for all three compounds. Response factors of 0.00022, 0.00012, and 0.0020 were used for calculating basalin, CDNB, and dinocap recoveries, respectively. The response factor for dinocap was determined by using only one of the isomer peaks.

Standards of basalin, CDNB, and dinocap were obtained from the reference standards repository of EPA-HERL; a standard was also obtained for dinocap from a chemical supply house. Second standards were not obtained for basalin and CDNB; it was not possible to check the purity of the standards obtained for basalin and CDNB from the reference standards repository. A 0.1 µg/mL standard solution of dinocap in hexane was prepared from the dinocap standard obtained from the chemical supply house; this solution was analyzed in duplicate and compared to equivalent standard solutions prepared from the dinocap obtained from the reference standards repository.

RESULTS AND DISCUSSION

The dinitro aromatic pesticides were not detected in unspiked reagent water or POTW effluent samples. The chromatograms obtained from reagent water and POTW effluent blanks contained other small peaks. The patterns displayed by these peaks were similar regardless of the sample matrix. The

TABLE 20. DINITRO AROMATIC PESTICIDES CALIBRATION DATA

Compound	Concentration, μg/L[(a)]	Average Peak Area	Standard Deviation, %	Number of Replicates	Average Response Factors
Basalin	0.05	222	12	10	0.00023
Basalin	0.1	528	10	11	0.00019
Basalin	1.0	3890	5	11	0.00026
CDNB	0.05	463	4	11	0.00011
CDNB	0.1	993	5	10	0.00010
CDNB	1.0	7260	2	11	0.00014
Dinocap	0.1	50	22	8	0.0020
Dinocap	1.0	476	7	8	0.0021
Dinocap	1.5	789	4	3	0.0019

(a) Equivalent concentration of compound in water sample.

peaks may have been introduced during sample preparation. The interference peaks eluted in F1 of the Florisil cleanup. These peaks represented interferences in the low μg/L range and did not interfere with the determination of basalin and CDNB. One unidentified peak eluted within the retention time range of where the dinocap isomers eluted; quantification of dinocap was therefore based on one of the isomer peaks as shown in Figure 43.

Recoveries of basalin from reagent water and POTW effluent samples, before cleanup, were greater than 94 percent at both the 0.1 and 1.0 μg/L concentration levels. Recovery of basalin from reagent water at the 0.1 μg/L level was high, 138 percent; the reason for this high recovery was not apparent. In most cases, recoveries of basalin from reagent water and POTW effluent samples were not significantly changed when the Florisil cleanup procedure was used. Recovery of basalin at the 0.1 μg/L concentration level from POTW effluent dropped from 94 percent to 74 percent when the cleanup procedure was used. The reason for this was not apparent. Chromatograms obtained from the GC analyses of POTW effluent extracts, unspiked and spiked with basalin at the 1.0 μg/L concentration level, are shown in Figure 39. The same extracts, after Florisil cleanup, are shown in Figure 41. Basalin recovery data are given in Table 21.

Recoveries of CDNB from reagent water and POTW effluent samples were generally low, ranging from 69 to 91 percent. Recoveries of CDNB were not significantly changed when the Florisil cleanup step was used. Although

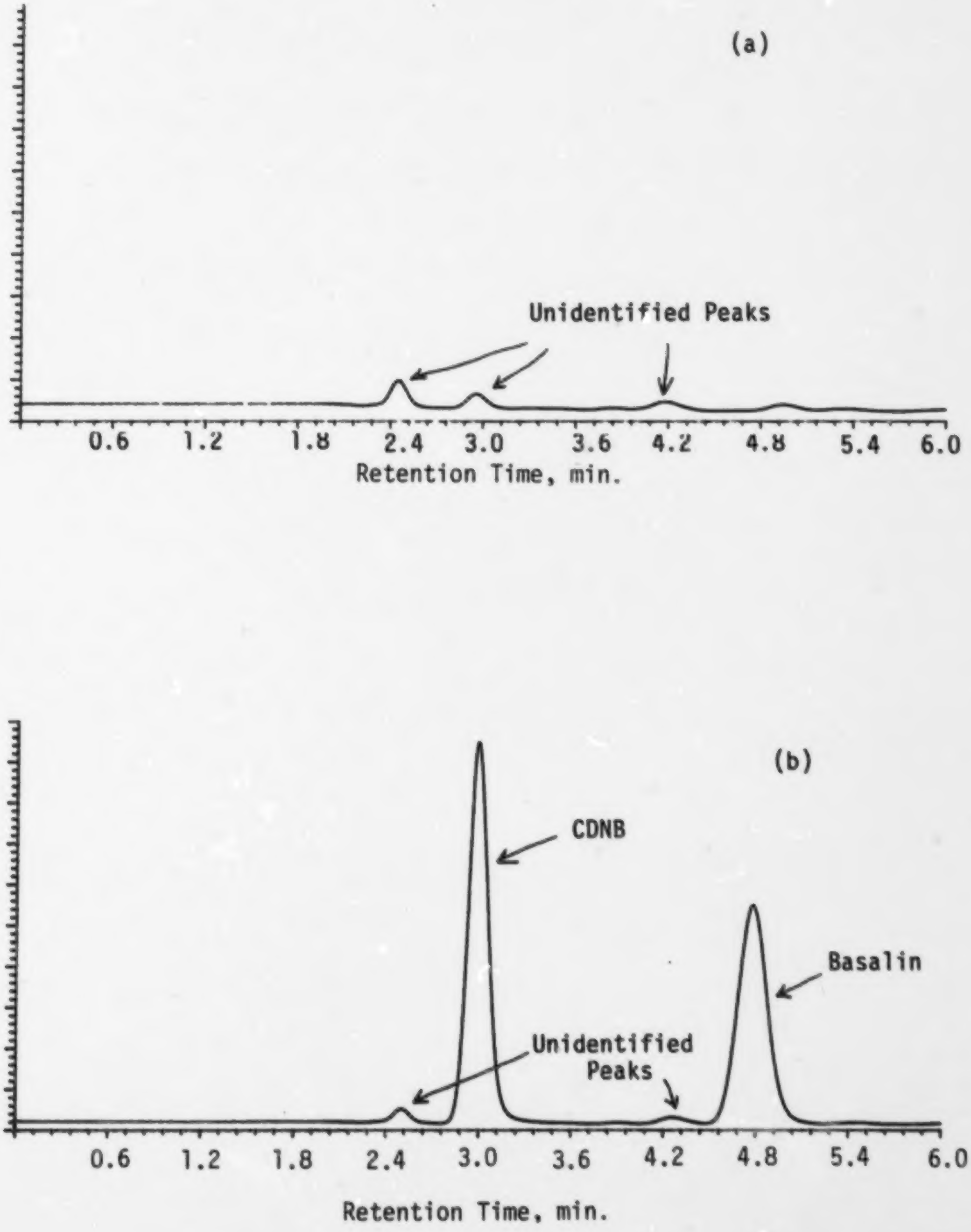


FIGURE 39. GC-ECD CHROMATOGRAM OF (a) POTW EFFLUENT AND (b) POTW EFFLUENT SPIKED WITH BASALIN AND CDNB AT THE 1.0 μg/L LEVEL, BEFORE CLEANUP.

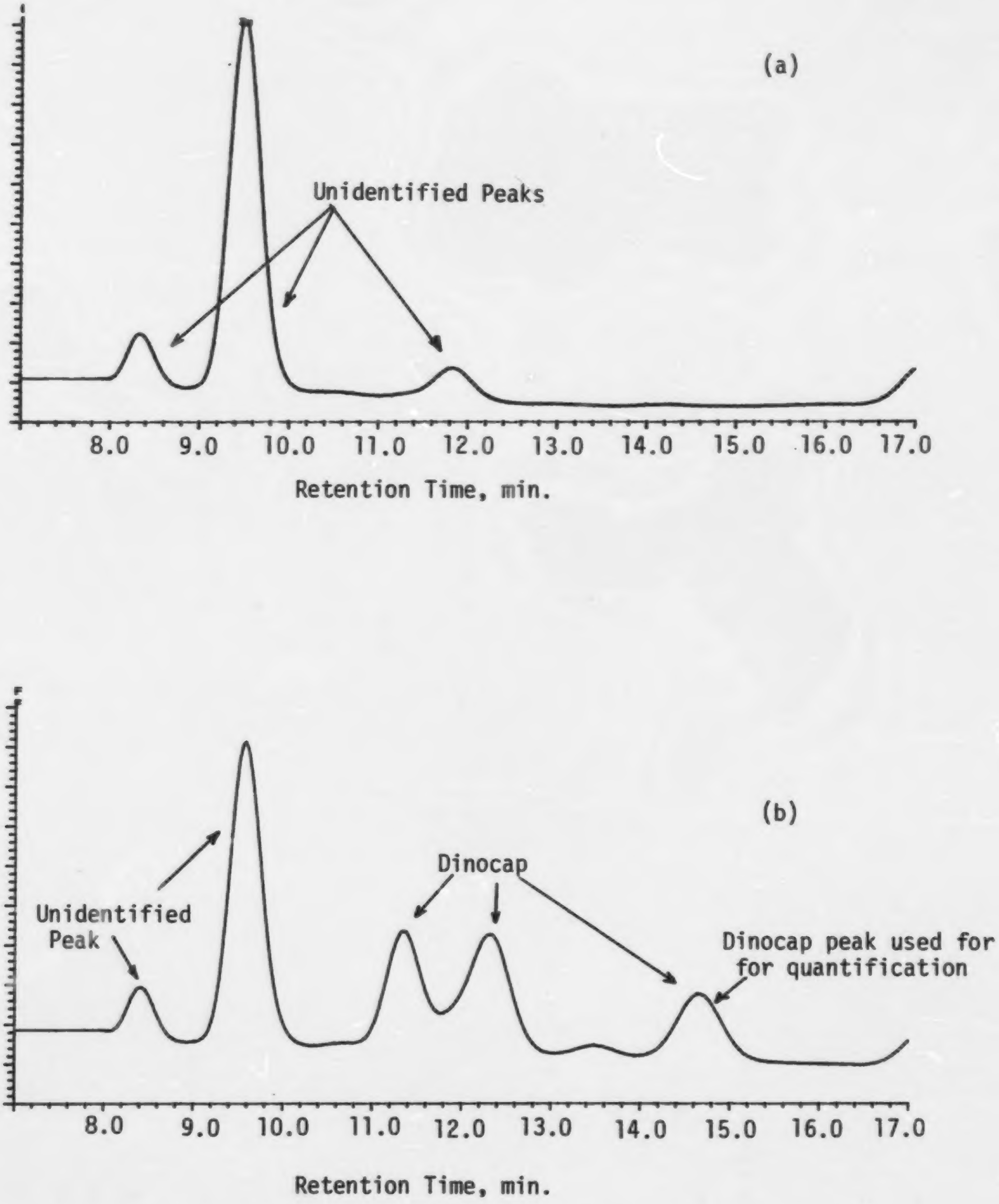


FIGURE 40. GC-ECD CHROMATOGRAM OF (a) POTW EFFLUENT, AND (b) POTW EFFLUENT SPIKED WITH DINOCAP AT THE 1.0 μg/L LEVEL, BEFORE CLEANUP.

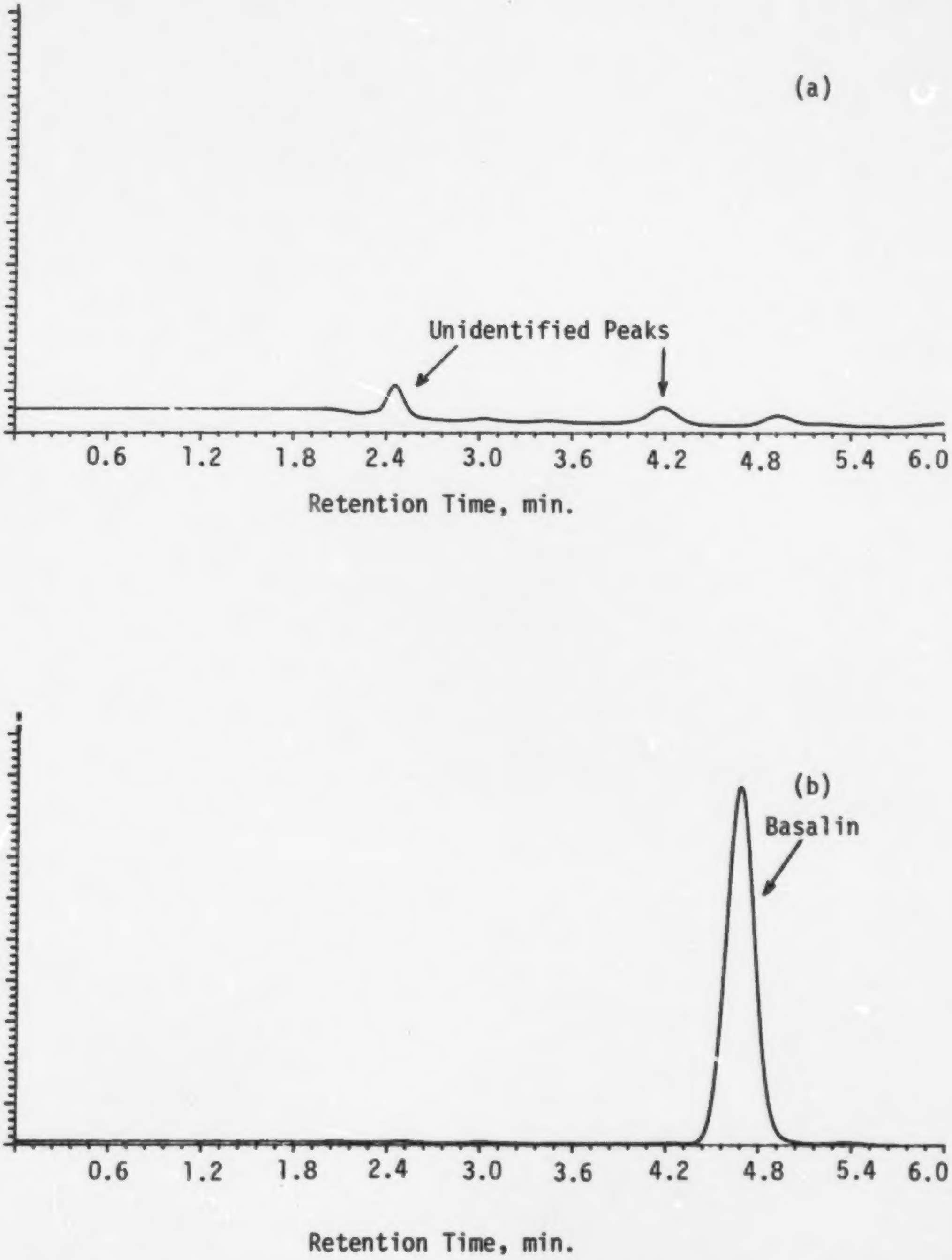


FIGURE 41. GC-ECD CHROMATOGRAM OF (a) POTW EFFLUENT AND (b) POTW EFFLUENT SPIKED WITH BASALIN AT THE 1.0 μg/L LEVEL, AFTER FLORISIL CLEANUP.

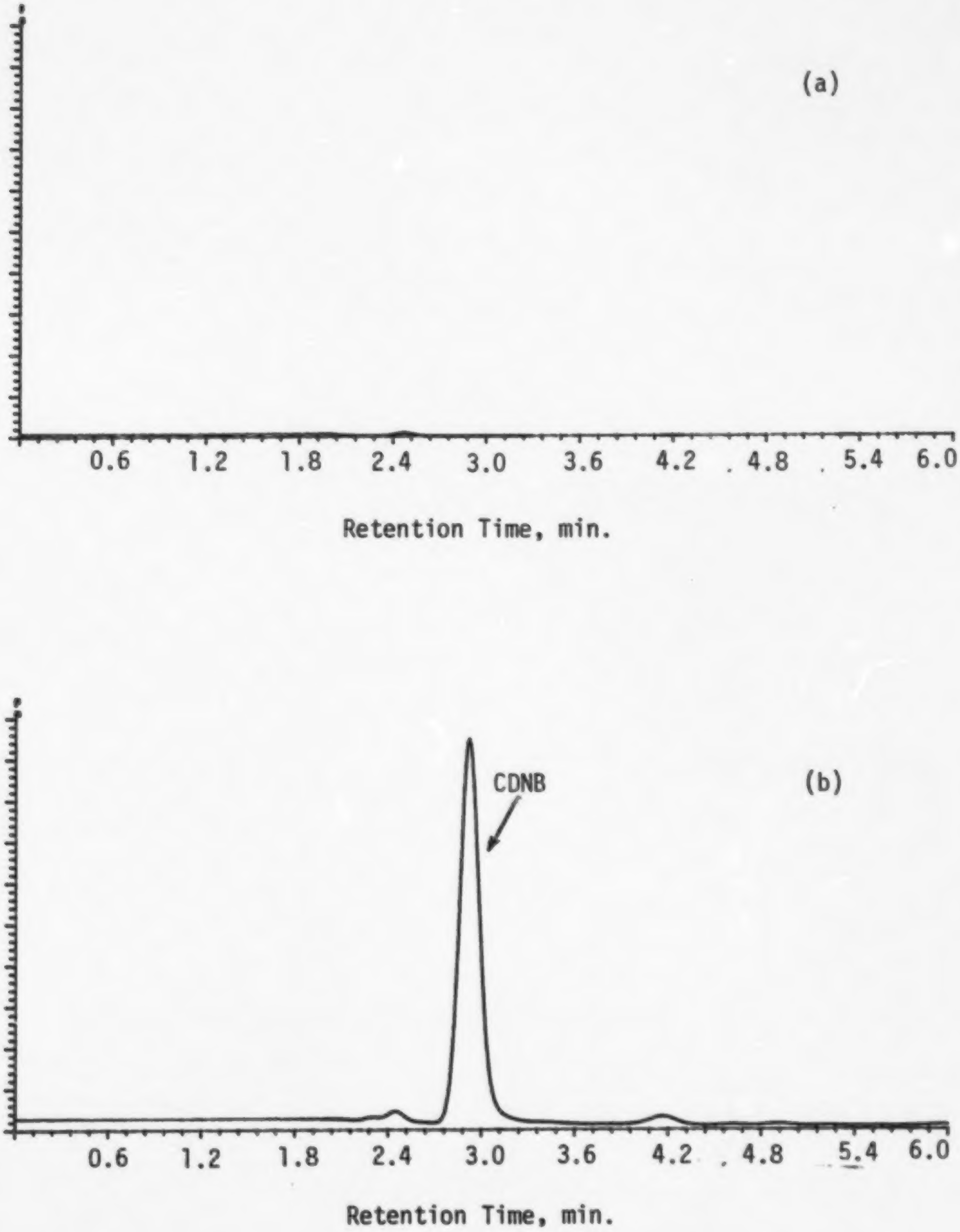


FIGURE 42. GC-ECD CHROMATOGRAM OF (a) POTW EFFLUENT, AND (b) POTW EFFLUENT SPIKED WITH CDNB AT THE 1.0 µg/L LEVEL, AFTER FLORISIL CLEANUP.

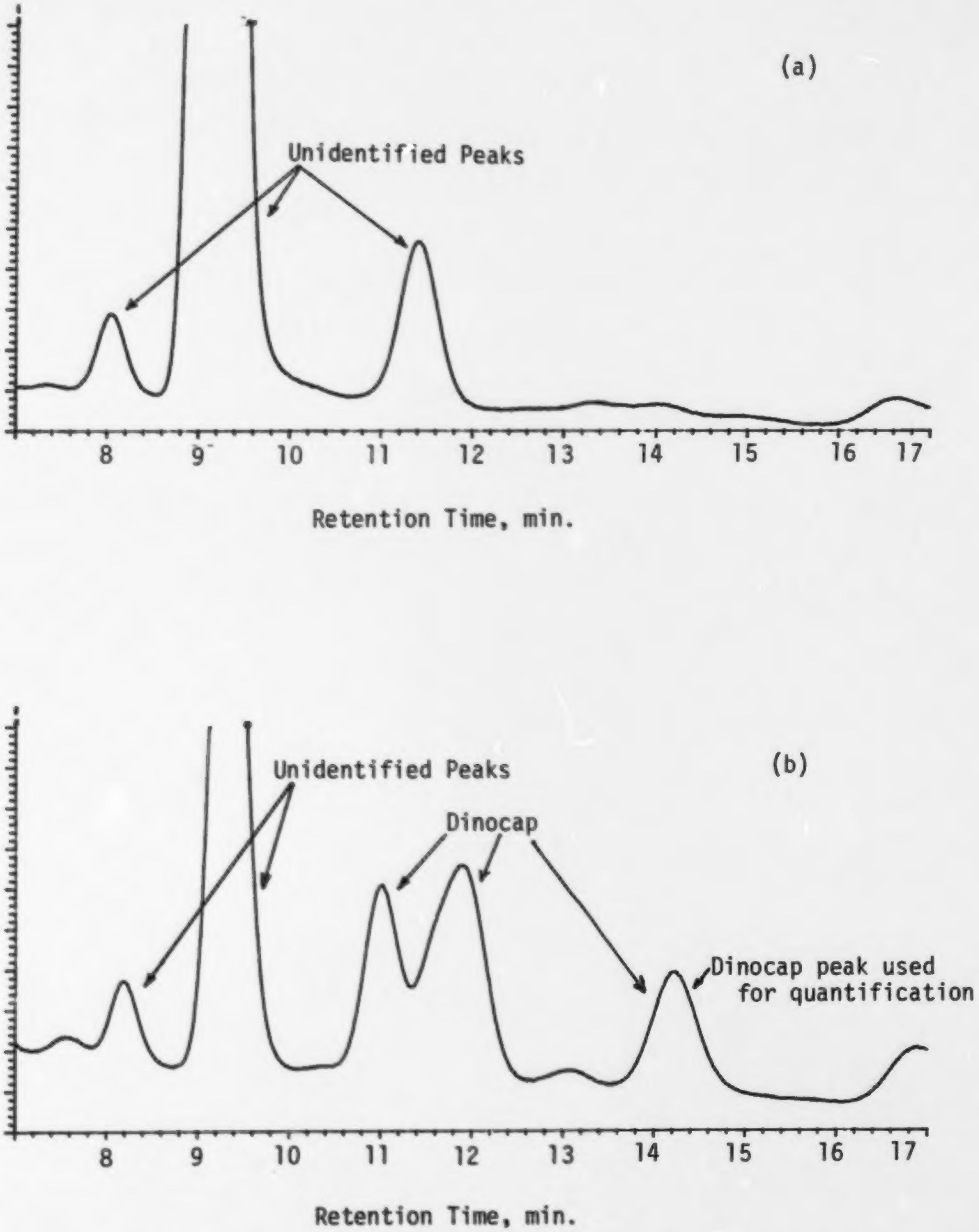


FIGURE 43. GC-ECD CHROMATOGRAM OF (a) POTW EFFLUENT, AND (b) POTW EFFLUENT SPIKED WITH DINOCAP AT THE 1.0 µg/L LEVEL, AFTER FLORISIL CLEANUP.

TABLE 21. RECOVERIES OF DINITRO AROMATIC PESTICIDES FROM WATER[c]

Compound	Spike Level, µg/L	Matrix[a]	Before Cleanup			After Cleanup		
			Blank Level	Recovery, %	Standard Deviation,%	Blank Level	Recovery, %	Standard Deviation,%
Basalin	0.1	1	ND[b]	130	12	ND	126	8
Basalin	1.0	1	ND	113	4	ND	109	10
Basalin	0.1	2	ND	94	6	ND	74	3
Basalin	1.0	2	ND	113	2	ND	108	3
CDNB	0.1	1	ND	91	8	ND	89	6
CDNB	1.0	1	ND	69	5	ND	71	5
CDNB	0.1	2	ND	78	2	ND	76	4
CDNB	1.0	2	ND	71	2	ND	70	2
Dinocap	0.1	1	ND	78	7	ND	26	3
Dinocap	1.0	1	ND	77	4	ND	72	14
Dinocap	0.1	2	ND	76	40	ND	123	53
Dinocap	1.0	2	ND	72	3	ND	80	10

(a) 1=Reagent water; 2=Columbus POTW secondary effluent.
(b) ND=not detected.
(c) Recovery data are averages of three replicate analyses.

the method stated that CDNB should elute in Florisil F1 (methylene chloride), CDNB eluted in Florisil F2 (10 percent acetone in methylene chloride). Chromatograms obtained from the GC analyses of POTW effluent extracts, unspiked and spiked with CDNB at the 1.0 µg/L concentration level, are shown in Figure 39. The same extracts, after Florisil cleanup, are shown in Figure 42. CDNB recovery data are given in Table 21.

Dinocap recovery studies were conducted at the 0.1 and 1.0 µg/L concentration levels. Data obtained from the 0.1 µg/L concentration levels were generally not repeatable and unreliable. This concentration level was very close to the EDL for dinocap, making identification and integration of dinocap peaks difficult. Recoveries of dinocap from samples spiked at the 1.0 µg/L concentration level were generally low, ranging from 72 to 77 percent. Recoveries of dinocap at this concentration level were not significantly changed when the Florisil cleanup step was used. Chromatograms obtained from the GC analyses of POTW effluent extracts, unspiked and spiked with dinocap at the 1.0 µg/L concentration level, are shown in Figure 40. The same extracts, after Florisil cleanup, are shown in Figure 43. Dinocap recovery data are given in Table 21.

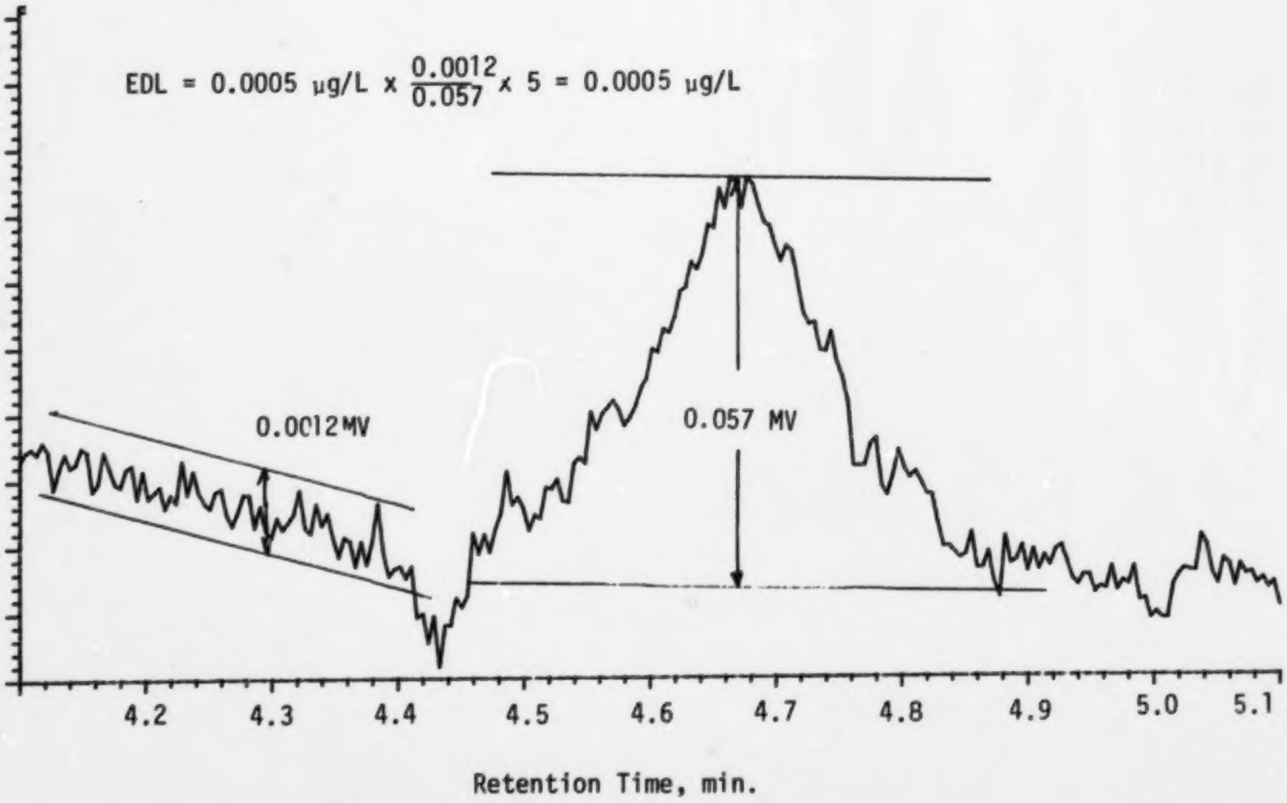
The EDLs for basalin and CDNB were determined by analyzing 0.0005 µg/mL standard solutions of basalin and CDNB in hexane; the resultant chromatograms are shown in Figures 44 and 45. This basalin solution yielded a S/N of 4.8. The EDL, defined as the concentration in water yielding a S/N equal to 5.0, was calculated to be 0.0005 µg/L of basalin. The CDNB solution yielded a S/N of 5.3. The EDL was calculated to be 0.0005 µg/L of CDNB. The EDL for dinocap was determined by analyzing a 0.05 µg/mL solution of dinocap in hexane; the resultant chromatogram is shown in Figure 46. This dinocap solution yielded a S/N of 1.9. The EDL was calculated to be 0.1 µg/L of dinocap. The EDL does not take into account any interferences or recovery losses that might be encountered from a particular matrix.

The average peak area of the 0.1 µg/mL standard solution of dinocap obtained from a chemical supply house was 22 percent lower than the average peak area of the same standard prepared from dinocap obtained from the reference standards repository. It can only be assumed that the dinocap obtained from the chemical supply house was not as pure as the supplier indicated.

RECOMMENDATIONS

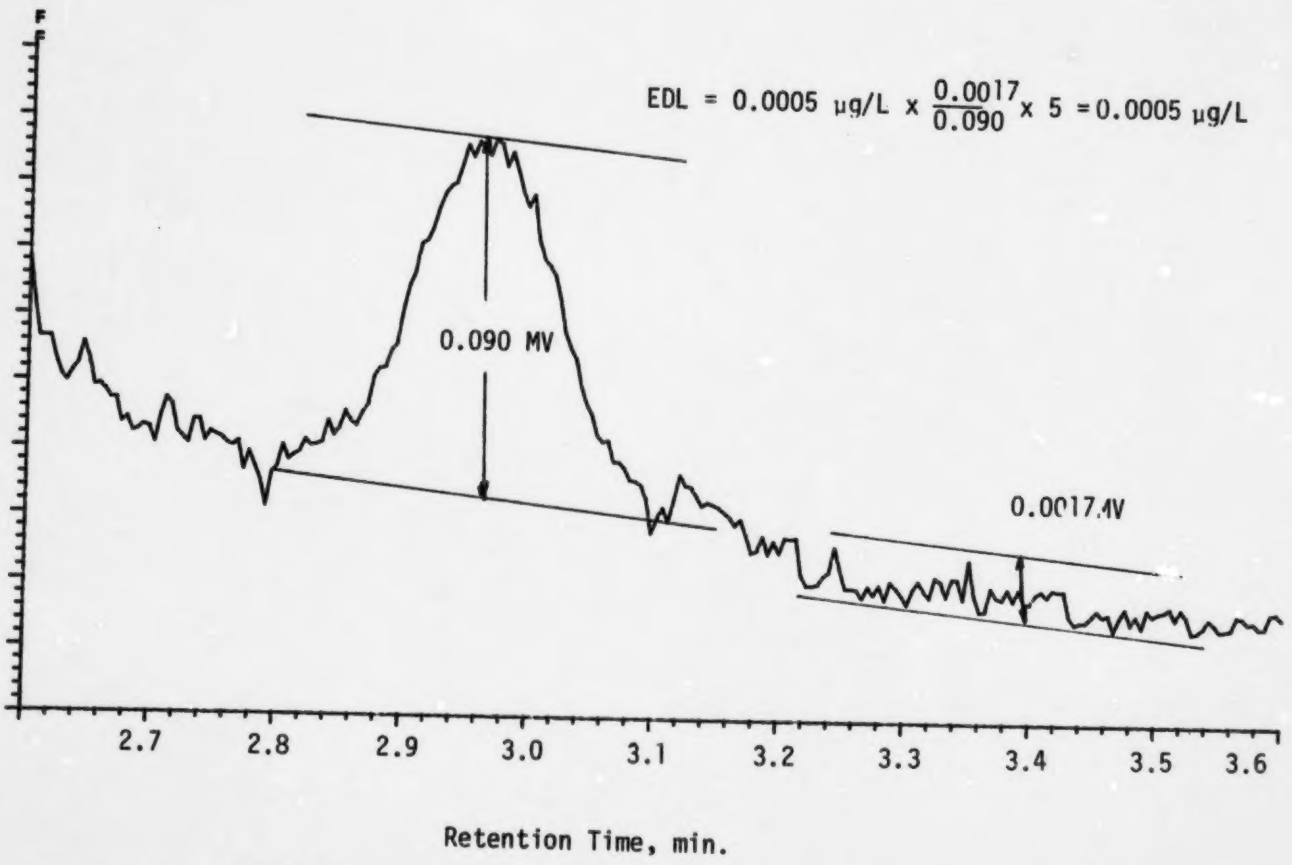
GC analysis conditions specified in the dinitro aromatic pesticides analysis method yielded excessively long elution times for all of the dinitro aromatic pesticides. The method should be modified by raising specified column temperatures as described earlier.

The analytical conditions and cleanup procedure specified in this method to determine the dinitro aromatic pesticides in water seem acceptable. However, recoveries of two of the compounds, CDNB and dinocap, are slightly low. This indicates that further study of compound long term stability in water and the efficiency of the extraction procedure may be warranted. Studies should be



$$EDL = 0.0005\ \mu g/L \times \frac{0.0012}{0.057} \times 5 = 0.0005\ \mu g/L$$

FIGURE 44. GC-ECD CHROMATOGRAM OF BASALIN STANDARD EQUIVALENT TO 0.0005 µg/L IN WATER USED TO DETERMINE METHOD EDL.



EDL = 0.0005 $\mu g/L \times \frac{0.0017}{0.090} \times 5 = 0.0005\ \mu g/L$

FIGURE 45. GC-ECD CHROMATOGRAM OF CDNB STANDARD EQUIVALENT TO 0.0005 μg/L IN WATER USED TO DETERMINE METHOD EDL.

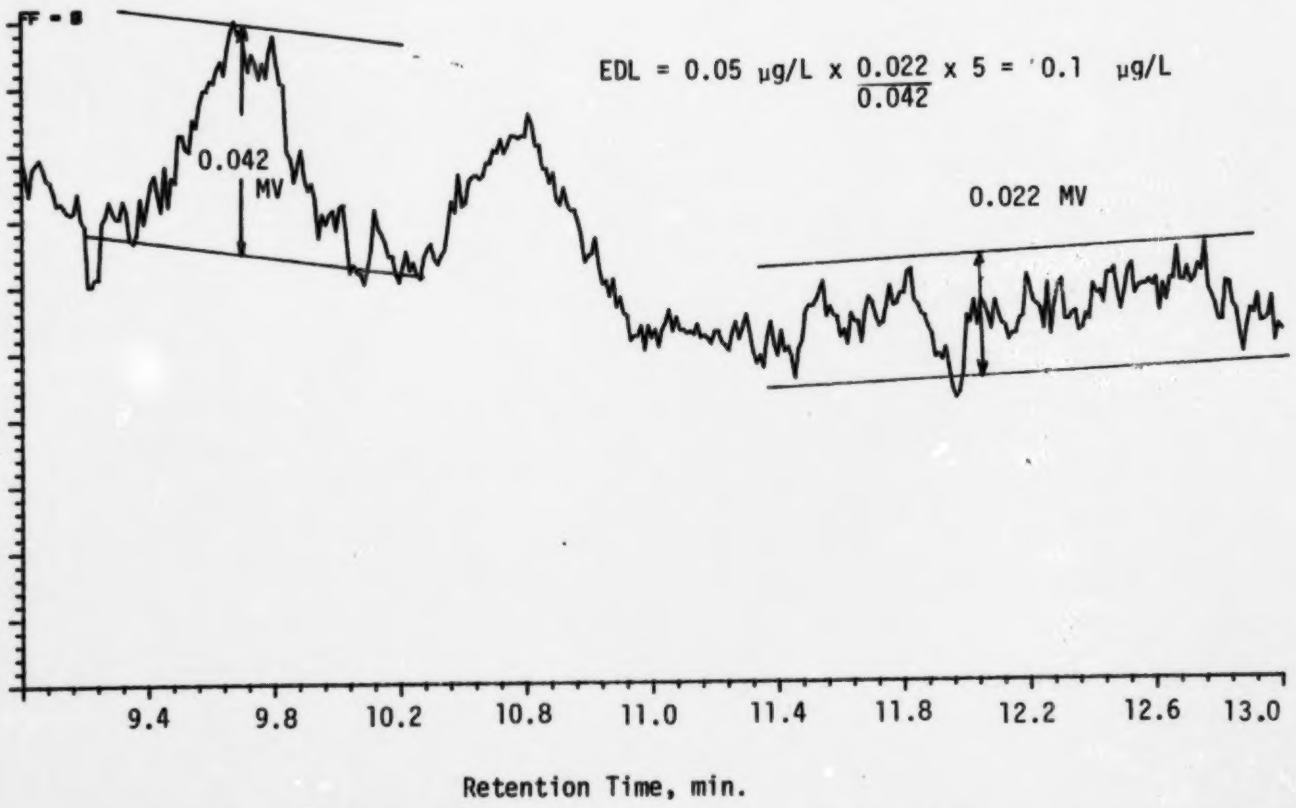


FIGURE 46. GC-ECD CHROMATOGRAM OF DINOCAP STANDARD EQUIVALENT TO 50 µg/L IN WATER USED TO DETERMINE METHOD EDL.

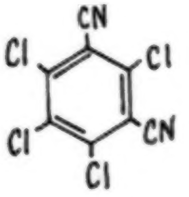
performed to determine the stability of CDNB and dinocap in water. It is possible that equilibrium between the two phases during extraction is not being achieved. Use of continuous extraction or perhaps tumbling might improve recovery efficiencies of the compounds. Tumbling is preferable to use of separatory funnels in this case since maximum mixing of the two phases is assured. Mixing achieved using separatory funnels is operator dependent.

SECTION 10

ANALYSIS OF ORGANOCHLORINE PESTICIDES IN WASTEWATER BY GAS CHROMATOGRAPHY

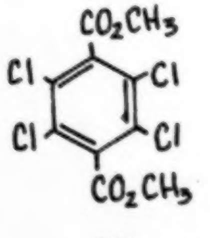
INTRODUCTION

Chlorothalonil (XXIV) is effective against a broad range of plant pathogens. The IUPAC name for chlorothalonil is tetrachloroisophthalonitrile and its CAS registry number is 1879-45-6. Common synonyms for chlorothalonil include Bravo, Daconil 2787, and Exotherm Termil. Chlorothalonil forms colorless crystals with a melting point of 250-251°C. Chlorothalonil has a solubility in water of 0.6 mg/kg at 25°C and is soluble in most organic solvents. Chlorothalonil is stable to acid, base and light. The acute oral LD50 for rats for chlorothalonil is >10,000 mg/kg (86).



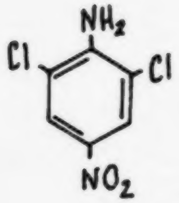
XXIV

DCPA (XXV) is a preemergent herbicide. The IUPAC name for DCPA is dimethyl tetrachloroterepthalate and its CAS registry number is 1861-32-1. Common synonyms for DCPA include chlorthal-dimethyl and Dacthal. DCPA forms colorless crystals with a melting point of 156°C. DCPA has a solubility in water of <0.5 mg/L at 25°C and is soluble in most organic solvents. The acute oral LD50 for DCPA is >3000 mg/kg (87).



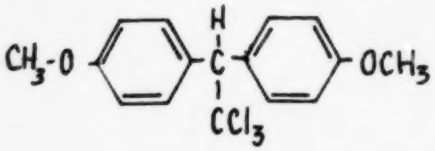
XXV

Dichloran (XXVI) is a fungicide. The IUPAC name for dichloram is 2,6-dichloro-4-nitroaniline and its CAS registry number is 99-30-9. Common synonyms for dichloran include Allisan, Botran, CDNA, AL-50, CNA, DCNA, dichloran, and Resistan. Dichloran forms yellow crystals with a melting point of 192-194°C. Dichloran is insoluble in water and moderately soluble in most organic solvents. The acute oral LD50 for dichloran for rats is 1500-4000 mg/kg.



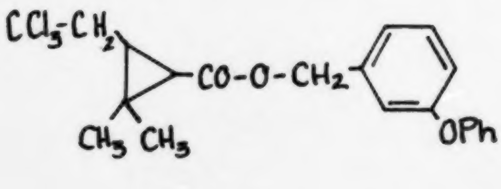
XXVI

Methoxychlor (XXVII) is a nonsystemic contact and stomach insecticide. The IUPAC name for methoxychlor is 1,1,1-trichloro-2,2-bis(4-methoxyphenyl)-ethane and its CAS registry number is 72-43-5. A common synonym for methoxychlor is Marlate. Methoxychlor is a grey flaky powder which is practically insoluble in water and is soluble in most organic solents. Methoxychlor is resistant to oxidation and heat. The acute oral LD50 for rats for methoxychlor is 6000 mg/kg (88).



XXVII

Permethrin (XXVIII) is a contact insecticide. The IUPAC name for permethrin is 3-phenoxybenzyl(1RS)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropanecarboxylate and its CAS registry number is 52645-53-1. Technical permethrin is a yellowish-brown liquid which partially crystallizes



XXVIII

at ambient temperatures and is completely liquid at temperatures greater than 60°C. Permethrin has a solubility in water of 0.1 mg/L at 20°C and is soluble in most organic solvents. Permethrin degrades in light and is more stable in acid than in base. The oral LD50 for permethrin in rats is 430-4000 mg/kg, the exact number depending upon the cis/trans ratio of the permethrin (89).

Analysis methods for the organochlorine pesticides in various matrices have been reported. These methods used GC coupled with EC and Hall detectors (24,35,51,52,53,58,90,91,92,93,94,95). An HPLC method for the determination of chlorothalonil and permethrin is also reported (33).

The method provided by the Project Officer for evaluation for determination of organochlorine pesticides consisted of adjustment of the sample pH to the range of 5 to 9 by addition of dilute sodium hydroxide or sulfuric acid; extraction of the sample with methylene chloride using a separatory funnel; drying of the sample using anhydrous sodium sulfate; concentration of the sample to 10 mL after exchanging the solvent with hexane; and analysis of the sample extract by packed column GC-ECD. A cleanup procedure using Florisil for samples containing DCPA and dichloran is included in the method. A cleanup procedure using 3 percent deactivated silica gel for samples containing chlorothalonil, methoxychlor, and permethrin was included in the method.

CONCLUSIONS

A method designed to determine organochlorine pesticides in wastewaters was evaluated. The extraction, cleanup, and analysis portions of the method were found to be acceptable for determination of cis- and trans-permethrin in reagent water and POTW effluent. Recoveries of chlorothalonil and dicloran from water could not be determined because of the presence of interferences. The method may not be applicable to samples containing chlorothalonil and dicloran at concentration levels less than 1 to 10 µg/L because of the likelihood of the presence of interferences. Recoveries of DCPA and methoxychlor were concentration and/or matrix dependent. The method was acceptable at the higher spiking level of 0.2 µg/L for DCPA and 10 µg/L for methoxychlor with recoveries being generally greater than 75 percent.

EXPERIMENTAL

The following procedure was outlined in the organochlorine pesticide analysis method:

1. Place sample (approximately 1 liter) into a 2-liter separatory funnel, and adjust the pH of the sample to within the range of 5 to 9 with dilute sodium hydroxide or sulfuric acid.

2. Extract the sample with three 60-mL portions of methylene chloride and combine the extracts.

3. The combined extracts are dried by pouring through a chromatography column containing 10 cm of anhydrous sodium sulfate.

4. Concentrate the combined extracts to an apparent volume of 1 mL using K-D equipment with the water bath at 80 to 85°C. The extracts are reconcentrated to 10 mL after the addition of 50 mL of hexane using K-D equipment with the water bath at 90 to 95°C.

5. A Florisil cleanup procedure is used for samples that contain DCPA and dichloran, and that require cleanup. A specified amount of Florisil, determined by its lauric acid value, is placed in a chromatography column. The sample is applied to the column in hexane, and the column is eluted with 200 mL of 6 percent ethyl ether in hexane, which is discarded. The column is then eluted with 200 mL of 15 percent ethyl ether in hexane. This fraction contains DCPA and dichloran. A cleanup procedure using 3 percent deactivated silica gel is used for samples that contain chlorothalonil, methoxychlor, and the permethrins, and that require cleanup. Silica gel, 3.5 grams, is placed in a chromatography column. The sample is applied to the column in hexane, and the column is eluted with 25 mL of hexane and 25 mL of 6 percent methylene chloride in hexane, both of which are discarded. The column is then eluted with 25 mL of 25 percent methylene chloride in hexane. This fraction contains chlorothalonil, methoxychlor, and the permethrins. The fractions obtained from the Florisil and silica gel cleanup procedures are concentrated to 10 mL after the addition of 50 mL of hexane using K-D equipment with the water bath at 90 to 95°C.

6. Analyze the sample by GC-ECD using the following conditions:
   - 180 cm long by 2 mm ID glass column packed with 1.5 percent OV-17/1.95 percent OV-210 on Chromosorb W-HP (100/120 mesh);
   - 5 percent methane/95 percent argon carrier gas at 30 mL/min flow rate;
   - oven temperature of 200°C isothermal for chlorothalonil, DCPA, dicloran, and methoxychlor, and 220°C isothermal for the permethrins;
   - injection volume of 5 μL.

The EDLs for the dinitro aromatic pesticides originally listed in the method were 0.002 μg/L for chlorothalonil, DCPA, and trans-permethrin, and 0.001 μg/L for dicloran, methoxychlor, and cis-permethrin. The EDLs for methoxyclor and the permethrins were estimated to be too low; EDLs of 0.1 μg/L were considered to be more likely. After telephone consultation with the EPA Task Officer, it was agreed that method evaluations would be done using spike levels of 1.0 and 10 μg/L for methoxychlor, cis-permethrin, and trans-permethrin.

Samples processed included the following:

1. Triplicate reagent water samples. Reagent water was obtained from a Millipore system.

2. Triplicate reagent water samples spiked with chlorothalonil and DCPA at the 0.02 μg/L concentration level, dicloran at the 0.01 μg/L concentration level, and methoxychlor, cis-permethrin, and trans-permethrin at the 1 μg/L concentration level.

3. Triplicate reagent water samples spiked with chlorothalonil and DCPA at the 0.2 μg/L concentration level, dicloran at the 0.1 μg/L concentration level, and methoxychlor, cis-permethrin, and trans-permethrin at the 10 μg/L concentration level.

4. Triplicate POTW secondary effluent samples. POTW secondary effluent was obtained from the City of Columbus.

5. Triplicate POTW secondary effluent samples spiked with chlorothalonil and DCPA at the 0.02 μg/L concentration level, dicloran at the 0.01 μg/L concentration level, and methoxychlor, cis-permethrin, and trans-permethrin at the 1 μg/L concentration level.

6. Triplicate POTW secondary effluent samples spiked with chlorothalonil and DCPA at the 0.2 μg/L concentration level, dicloran at the 0.1 μg/L concentration level, and methoxychlor, cis-permethrin, and trans-permethrin at the 10 μg/L concentration level.

A second set of samples as described in points 1-6 were prepared and divided in half; each half was treated with either silica gel or Florisil cleanup procedures and reanalyzed by GC-ECD. Attempts were made to obtain recovery data for all compounds both after silica gel and Florisil cleanup procedures. All fractions were analyzed for all of the dinitro aromatic compounds.

Recoveries were determined by comparison to standard solutions of chlorothalonil and DCPA prepared at the 1.0, 5.0, and 20 μg/L concentration levels, dicloran prepared at the 0.5, 2.5, and 10 μg/L concentration levels, and methoxychlor, cis-permethrin, and trans-permethrin prepared at the 50, 200, and 1500 μg/L concentration levels in hexane. These concentration levels were equivalent to 0.01, 0,05, and 0.2 μg/L of chlorothalonil and DCPA, 0.005, 0.025, and 0.1 μg/L of dicloran, and 0.5, 2.0, and 15 μg/L of methoxychlor, cis-permethrin, and trans-permethrin in the original samples assuming a final extract volume of 10 mL. Response factors were calculated for the standards and used to determine concentration levels in the above-mentioned reagent water and POTW effluent samples.

The method EDLs, defined as the concentration of each organochlorine pesticide in a sample yielding a S/N of 5, were determined by injecting 0.25 µg/L standard solutions of chlorthalonil, dichloran, and DCPA, 10 µg/L standard solution of cis-permethrin and trans-permethrin, and a 5.0 µg/L solution of methoxychlor prepared in hexane. The solutions were equivalent to 0.0025 µg/L concentrations of chlorothalonil, dichloran, and DCPA, 0.1 µg/L concentrations of cis-permethrin and trans-permethrin, and 0.05 µg/L concentration of methoxychlor in a water sample.

QUALITY ASSURANCE

Instrumentation was set up as described in the method. Sensitivity achieved was approximately that reported in the method for chlorothalonil, DCPA, and dicloran. As expected, sensitivity achieved was approximately one order of magnitude lower for methoxychlor and two orders of magnitude lower for cis-permethrin and trans-permethrin. Calibration standards consisted of solutions of chlorothalonil and DCPA prepared at the 1.0, 5.0, and 20 µg/L concentration levels, dicloran prepared at the 0.5, 2.5, and 10 µg/L concentration levels, and methoxychlor, cis-permethrin, and trans-permethrin prepared at the 50, 200, and 1500 µg/L concentration levels in hexane. Each calibration standard was analyzed in duplicate prior to analysis of any evaluation samples. A selected calibration standard was then analyzed after every five evaluation samples. Response factors were calculated for each calibration run by dividing the equivalent pesticide concentration level in the sample in µg/L by the pesticide peak area. Resultant calibration data are reported in Table 22. Response factors were repeatable over the entire calibration range for all of the organochlorine pesticides. Response factors used for quantification of chlorothalonil, DCPA, dichloran, methoxychlor, cis-permethrin, and trans-permethrin were 0.0058, 0.0053, 0.0056, 0.018, 0.11, and 0.089, respectively.

Standards of all six organochlorine pesticides were obtained from two independent sources. Calibration standards used for quantification of these compounds in the water samples were prepared from compounds obtained from the reference standards repository of EPA-HERL. Standard solutions of the six organochlorine pesticides were also prepared from compounds obtained from a chemical supply house; these solutions were analyzed in duplicate and compared to equivalent standard solutions prepared from the compounds obtained from the reference standards repository.

RESULTS AND DISCUSSION

Several unidentified peaks appeared in both spiked and unspiked reagent water and POTW extracts. Some of these peaks coeluted with chlorothalonil and dicloran and precluded recovery determinations for these two pesticides. In most cases, the Florisil or silica gel cleanup procedures removed some of the interfering peaks. However, enough of the peaks remained to make recovery determinations for chlorothalonil and dicloran impossible. It is unlikely that these peaks were actually chlorothalonil and dicloran since they were partially removed by the cleanup procedures. The source of these interferences was not identified. Significant problems due to interferences

TABLE 22. ORGANOCHLORINE PESTICIDES CALIBRATION DATA

Compound	Concentration, µg/L[(a)]	Average Peak Area	Standard Deviation, %	Number of Replicates	Average Response Factors
Chlorothalonil	0.01	1.73	21	8	0.0058
Chlorothalonil	0.05	8.46	19	7	0.0059
Chlorothalonil	0.20	35.6	14	8	0.0056
DCPA	0.01	1.68	14	8	0.0060
DCPA	0.05	10.3	4	8	0.0048
DCPA	0.2	40.0	4	8	0.0050
Dicloran	0.005	0.80	12	8	0.0063
Dicloran	0.025	4.55	7	8	0.0055
Dicloran	0.1	19.4	4	8	0.0052
Methoxychlor	0.5	25.4	16	8	0.020
Methoxychlor	2.0	117	15	7	0.017
Methoxychlor	15	858	6	8	0.017
Cis-permethrin	0.5	3.9	20	6	0.13
Cis-permethrin	2.0	18.5	13	8	0.11
Cis-permethrin	15	142	10	8	0.11
Trans-permethrin	0.5	5.54	19	5	0.090
Trans-permethrin	2.0	22.5	7	4	0.089
Trans-permethrin	15	170	4	4	0.088

(a) Equivalent concentration of compound in water sample.

were not observed for the other four compounds. Chromatograms obtained from the analyses of unspiked reagent water and POTW water samples, both before and after cleanup, are shown in Figures 47-51. Chromatograms obtained from the analyses of POTW effluent samples, unspiked and spiked with chlorothalonil at the 0.2 µg/L and dicloran at the 0.1 µg/L concentration levels are shown in Figure 47. The Florisil cleanup procedure was used for samples containing dicloran and the silica gel cleanup procedure was used for samples containing chlorathalonil. Chromatograms obtained from the same samples after silica gel or Florisil cleanup are shown in Figures 49 and 50. Data indicating the levels of interfering peaks found in the sample extracts are given in Table 23.

Recoveries of 0.02 µg/L levels of DCPA from reagent water were 128 ± 10 percent before Florisil cleanup and 73 ± 25 percent after cleanup. Recoveries of 0.2 µg/L levels of DCPA from reagent water before and after Florisil cleanup were more repeatable, 94 ± 11 percent and 84 ± 11 percent, respectively. These data may indicate that the reliability of the method is concentration dependent.

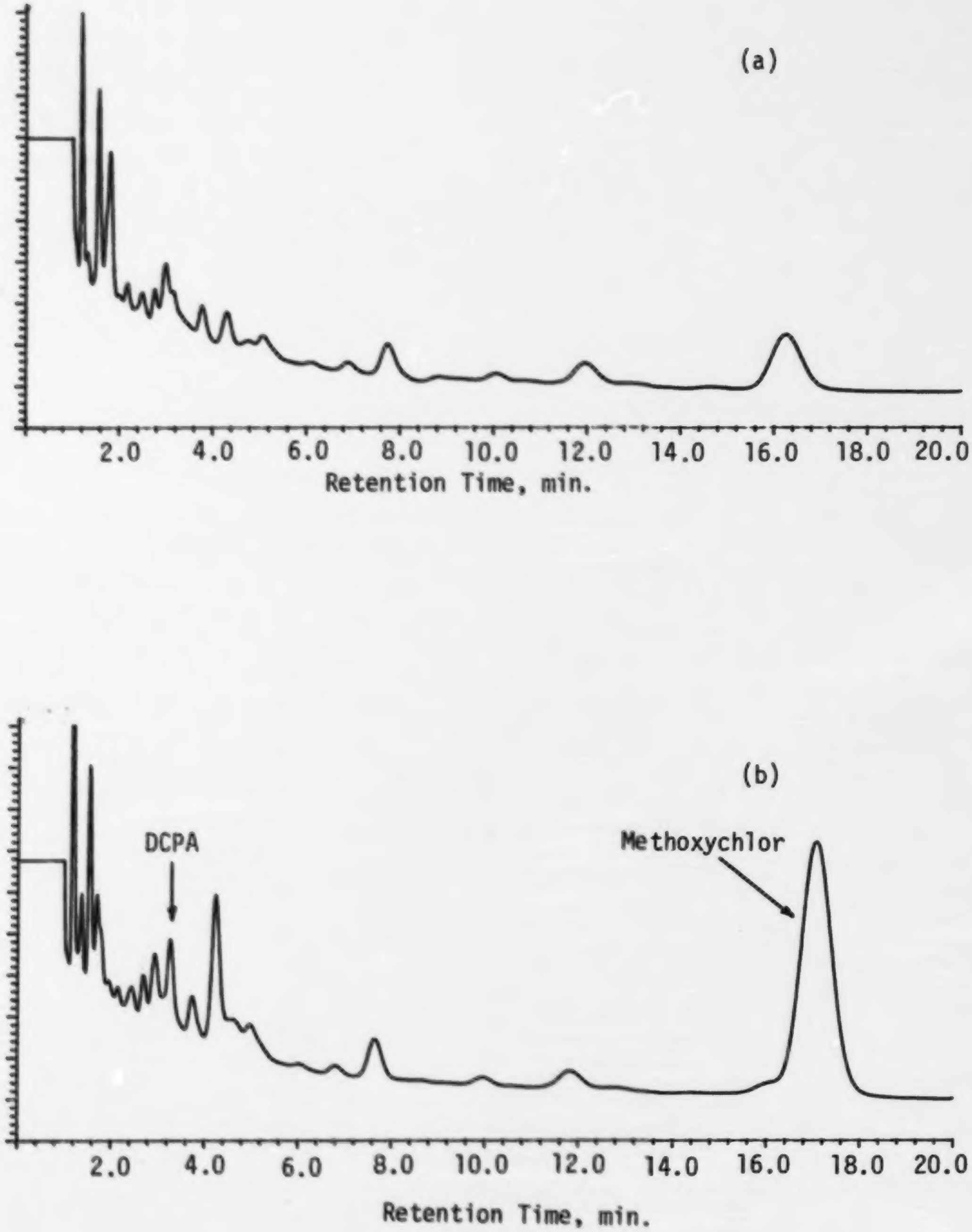


FIGURE 47. GC-ECD CHROMATOGRAM OF (a) POTW EFFLUENT AND (b) POTW EFFLUENT SPIKED WITH 0.2 μg/L CHLOROTHALONIL, 0.2 μg/L DCPA, 0.1 μg/L DICLORAN, AND 10 μq/L METHOXYCHLOR.

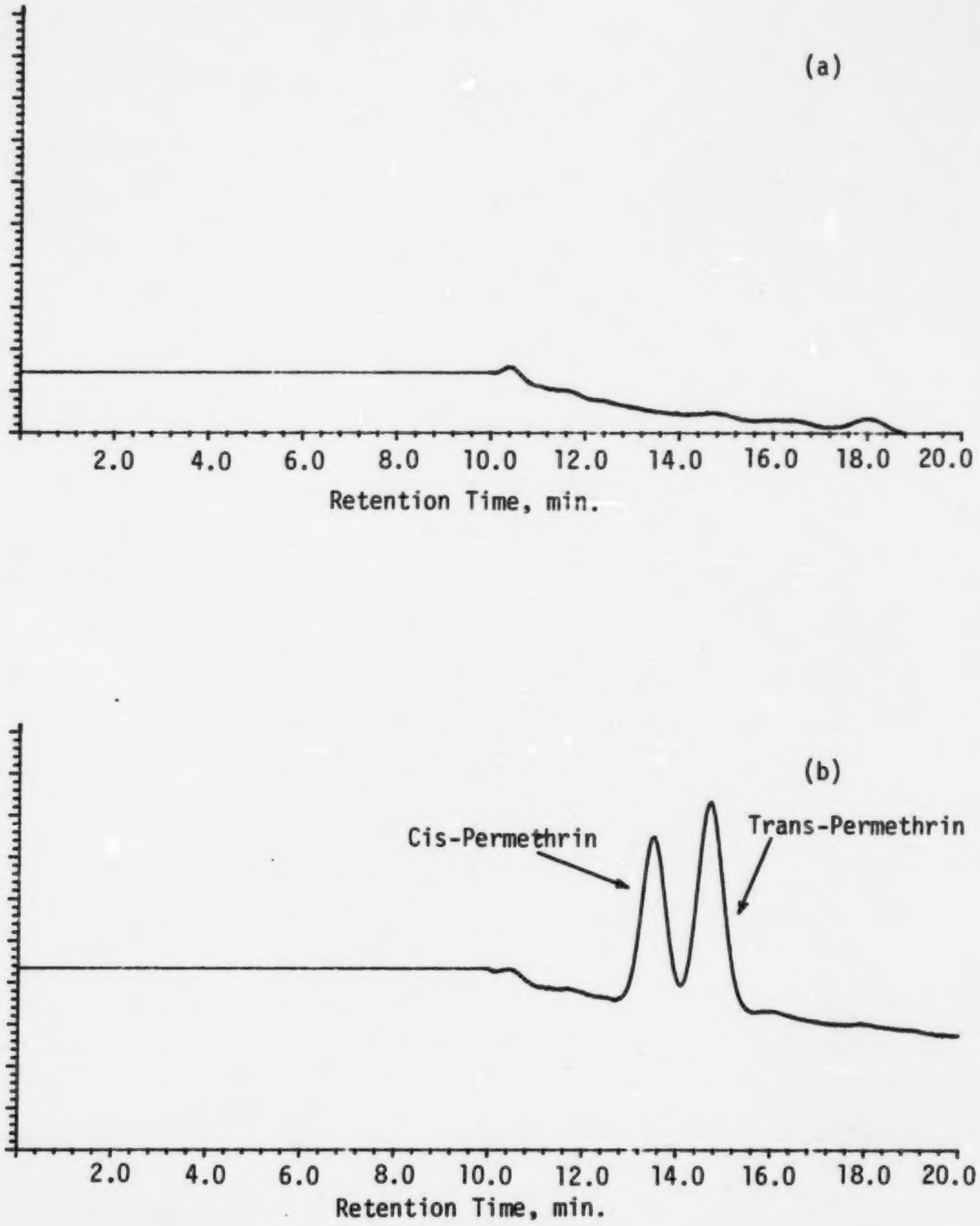


FIGURE 48. GC-ECD CHROMATOGRAM OF (a) POTW EFFLUENT, AND (b) POTW EFFLUENT SPIKED WITH CIS-PERMETHRIN AND TRANS-PERMETHRIN AT THE 10 μg/L LEVELS.

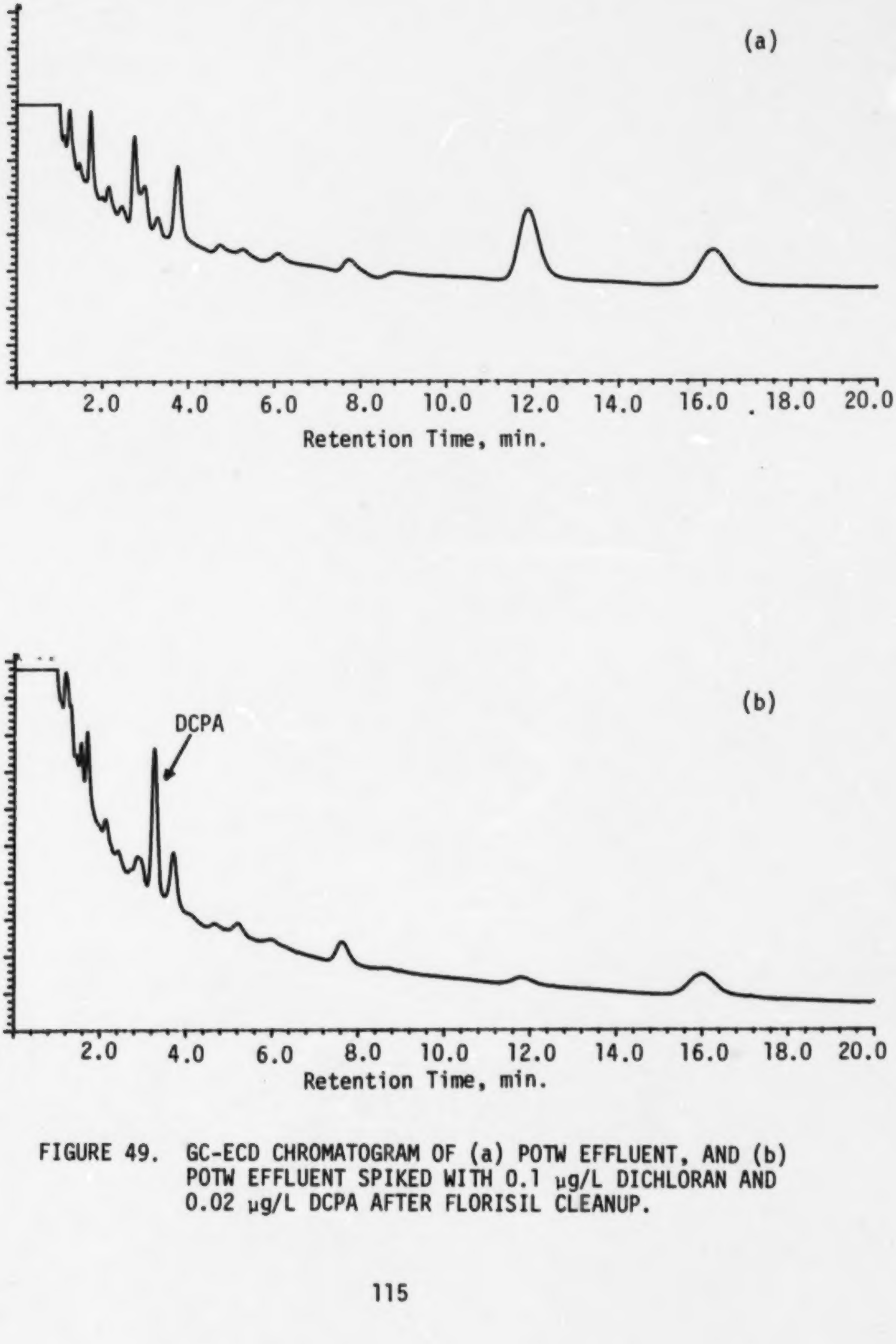


FIGURE 49. GC-ECD CHROMATOGRAM OF (a) POTW EFFLUENT, AND (b) POTW EFFLUENT SPIKED WITH 0.1 μg/L DICHLORAN AND 0.02 μg/L DCPA AFTER FLORISIL CLEANUP.

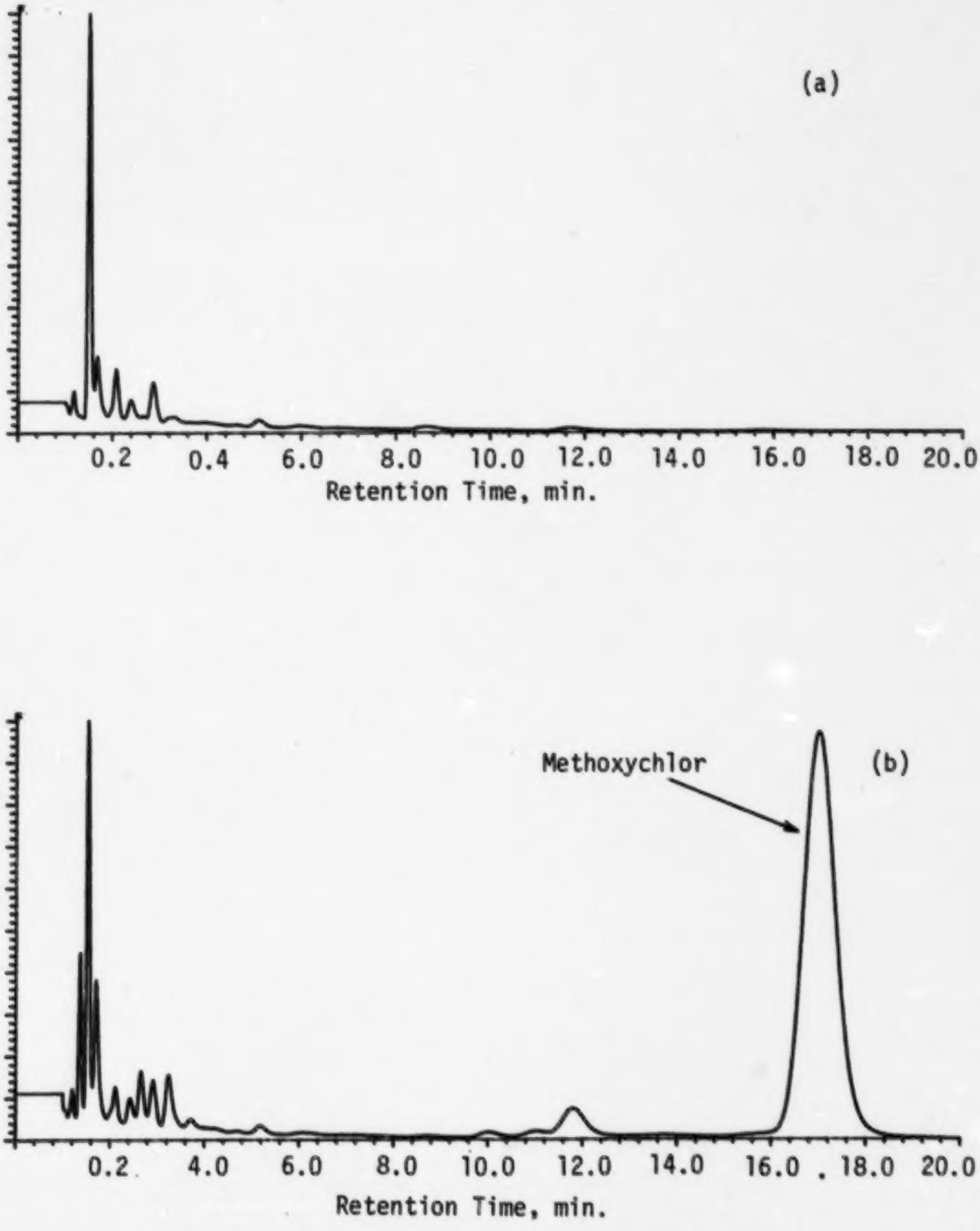


FIGURE 50. GC-ECD CHROMATOGRAM OF (a) POTW EFFLUENT, AND (b) POTW EFFLUENT SPIKED WITH 0.2 μg/mL CHLOROTHALONIL AND 10 μg/L METHOXYCHLOR AFTER SILICA GEL CLEANUP.

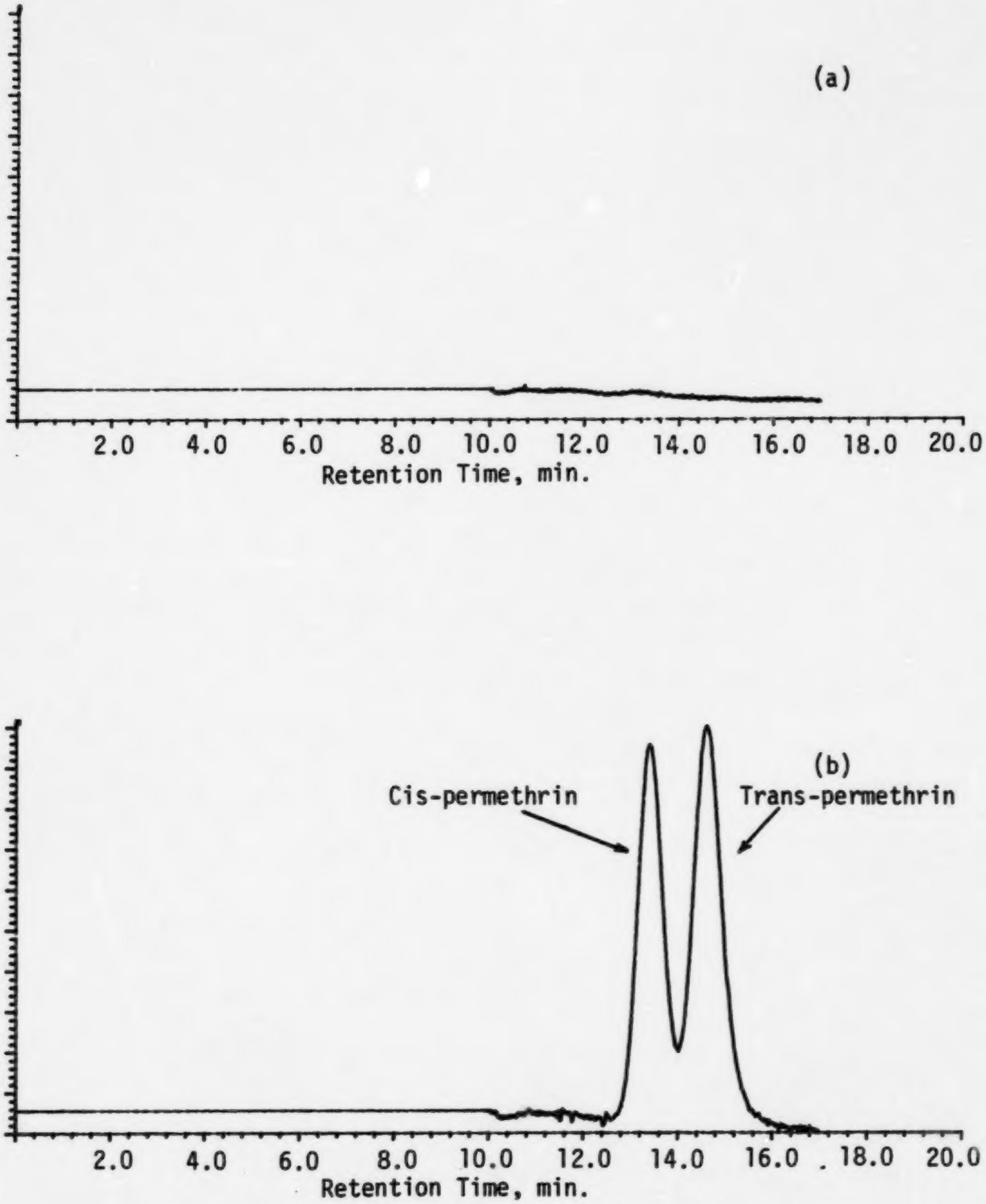


FIGURE 51. GC-ECD CHROMATOGRAM OF (a) POTW EFFLUENT, AND (b) POTW EFFLUENT SPIKED WITH CIS-PERMETHRIN AND TRANS-PERMETHRIN AT THE 10 μg/L LEVELS, AFTER SILICA GEL CLEANUP.

TABLE 23. RECOVERIES OF ORGANOCHLORINE PESTICIDES FROM WATER$^{(c)}$

Compound	Spike Level, µg/L	Matrix$^{(a)}$	Before Cleanup			After Cleanup		
			Blank Level	Recovery, %	Standard Dev., %	Blank Level	Recovery, %	Standard Dev.,%
Chlorothalonil	0.02	1	1.2	(d)	--	0.21	(d)	--
Chlorothalonil	0.2	1	1.2	(d)	--	0.21	(d)	--
Chlorothalonil	0.02	2	7.3	(d)	--	0.52	(d)	--
Chlorothalonil	0.2	2	7.3	(d)	--	0.52	(d)	--
DCPA	0.02	1	ND$^{(b)}$	128	18	ND	73 (51)$^{(e)}$	25 (42)
DCPA	0.2	1	ND	94	11	ND	84 (27)	11 (22)
DCPA	0.02	2	ND	ND	--	0.011	62 (46)	5 (57)
DCPA	0.2	2	ND	71	5	0.011	76 (40)	2 (4)
Dicloran	0.01	1	3.6	(d)	--	3.8	(d)	--
Dicloran	0.1	1	3.6	(d)	--	3.8	(d)	--
Dicloran	0.01	2	29	(d)	--	4.6	(d)	--
Dicloran	0.1	2	29	(d)	--	4.6	(d)	--
Methoxychlor	1.0	1	ND	57	1	ND	37	8
Methoxychlor	10	1	ND	106	12	ND	86	12
Methoxychlor	1.0	2	ND	47	12	ND	40	27
Methoxychlor	10	2	ND	99	2	ND	90	14
Cis-permethrin	1.0	1	ND	94	9	ND	91	6
Cis-permethrin	10	1	ND	91	2	ND	128	17
Cis-permethrin	1.0	2	ND	89	8	ND	97	14
Cis-permethrin	10	2	ND	85	3	ND	98	27
Trans-permethrin	1.0	1	ND	111	4	ND	83	12
Trans-permethrin	10	1	ND	98	3	ND	108	13
Trans-permethrin	1.0	2	ND	85	2	ND	41	11
Trans-permethrin	10	2	ND	90	2	ND	95	29

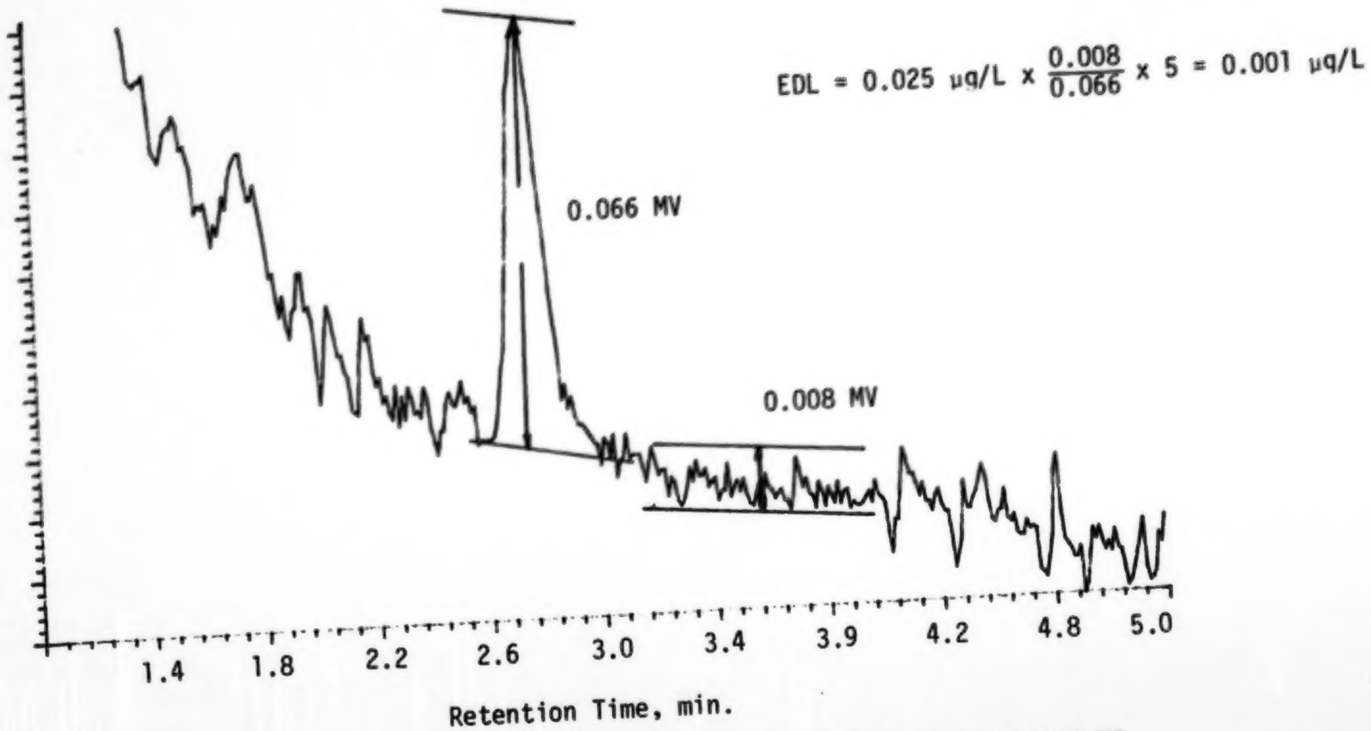
(a) 1=Reagent water; 2=Columbus POTW secondary effluent.
(b) ND = not detected.
(c) Recovery data are averages of three replicate analyses.
(d) Recovery data not calculated; compound was not detected because of interfering peaks.
(e) A Florisil cleanup procedure was specified for DCPA in method; data obtained for DCPA after silica gel cleanup are given in parentheses.

Recoveries of 0.02 µg/L levels of DCPA from POTW effluent before and after Florisil cleanup were 0 percent and 62 ± 4.7 percent, respectively. An interfering peak was introduced at the 0.01 µg/L level to these sample extracts during the Florisil cleanup procedure. This level is comparable to the amount of DCPA spiked into the POTW effluent. It is possible that 0.2 µg/L levels of DCPA are not recovered from the POTW effluents, and that the 62 percent recovery figure is due to some variability in the interfering compound peak area. Recoveries of 0.2 µg/L levels of DCPA from POTW effluent before and after Florisil cleanup were again more repeatable than those from the lower spiking level, 71 ± 5.0 percent and 76 ± 2.4 percent, respectively. These recoveries, however, are significantly lower than those from reagent water spiked at the 0.2 µg/L level with DCPA. DCPA recoveries seem to be not only concentration dependent but also matrix dependent. Recoveries of DCPA after silica gel cleanup were generally lower than those obtained from equivalent samples after Florisil cleanup. Chromatograms obtained from the analyses of unspiked POTW effluent and POTW effluent spiked with DCPA at the 0.2 µg/L level, prior to Florisil cleanup, are shown in Figure 47. Chromatograms obtained from the same samples after Florisil cleanup are shown in Figure 49. DCPA recovery data are given in Table 23.

Recoveries of methoxychlor spiked into reagent water or POTW effluent at the 1.0 µg/L levels were generally low, ranging from 37 to 57 percent. Recoveries increased to 86 to 106 percent when the methoxychlor spiking level was raised to 10 µg/L. The data indicate that recoveries of methoxychlor from water are concentration dependent. The silica gel cleanup procedure did not significantly affect methoxychlor recoveries. Methoxychlor was not detected in samples after Florisil cleanup. Chromatograms obtained from the analyses of unspiked POTW effluent and POTW effluent spiked with methoxychlor at the 10 µg/L level, prior to silica gel cleanup, are shown in Figure 47. Chromatograms obtained from the same samples after silica gel cleanup are shown in Figure 50. Methoxychlor recovery data are given in Table 23.

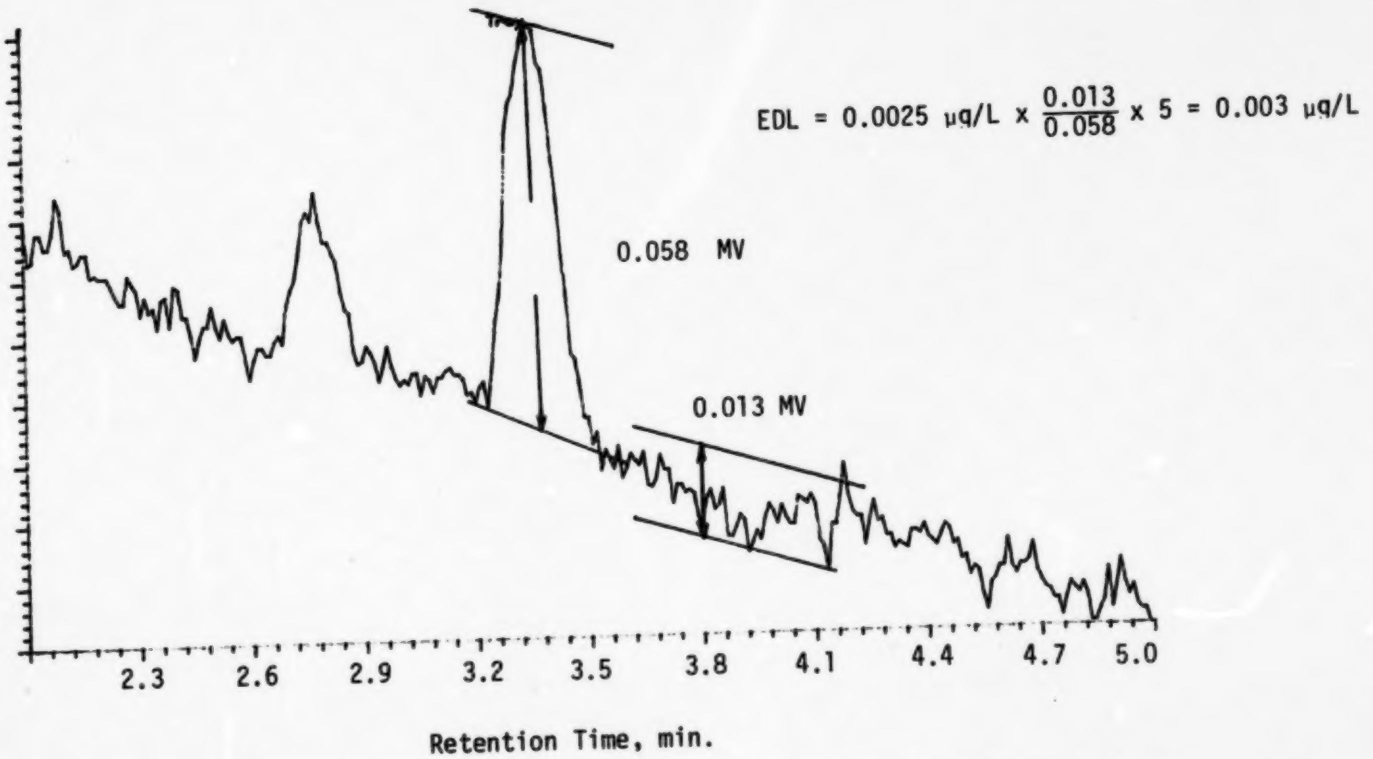
Recoveries of cis-permethrin and trans-permethrin were generally greater than 83 percent, regardless of the compound concentration level or sample matrix. The exception was the low recovery of 1.0 µg/L levels of trans-permethrin from reagent water. Only 41 percent of the trans-permethrin was recovered from these samples. The reason for this low recovery is not known. The data indicate that the silica gel cleanup procedure does not lead to significant losses of cis- or trans-permethrin. Records do not indicate a problem in sample preparation. Cis- and trans-permethrin were not detected in samples after Florisil cleanup. Chromatograms obtained from unspiked POTW effluent and POTW effluent spiked at the 10 µg/L level with cis- and trans-permethrin, before silica gel cleanup, are shown in Figure 48. Chromatograms obtained from the same samples after silica gel cleanup are shown in Figure 51. Recovery data for cis- and trans-permethrin are given in Table 23.

The EDLs for chlorothalonil, DCPA, and dicloran were determined by analyzing 0.25 µg/L standard solutions of these compounds in hexane; the resultant chromatograms are shown in Figures 52-54. The chlorothalonil solution yielded a S/N of 8.7. The EDL, defined as the concentration in water yielding a S/N equal to 5.0, was calculated to be 0.001 µg/L of chlorothalonil. The DCPA solution yielded a S/N of 4.5. The EDL was calculated to be 0.003 µg/L of DCPA. The dicloran solution yielded a S/N of



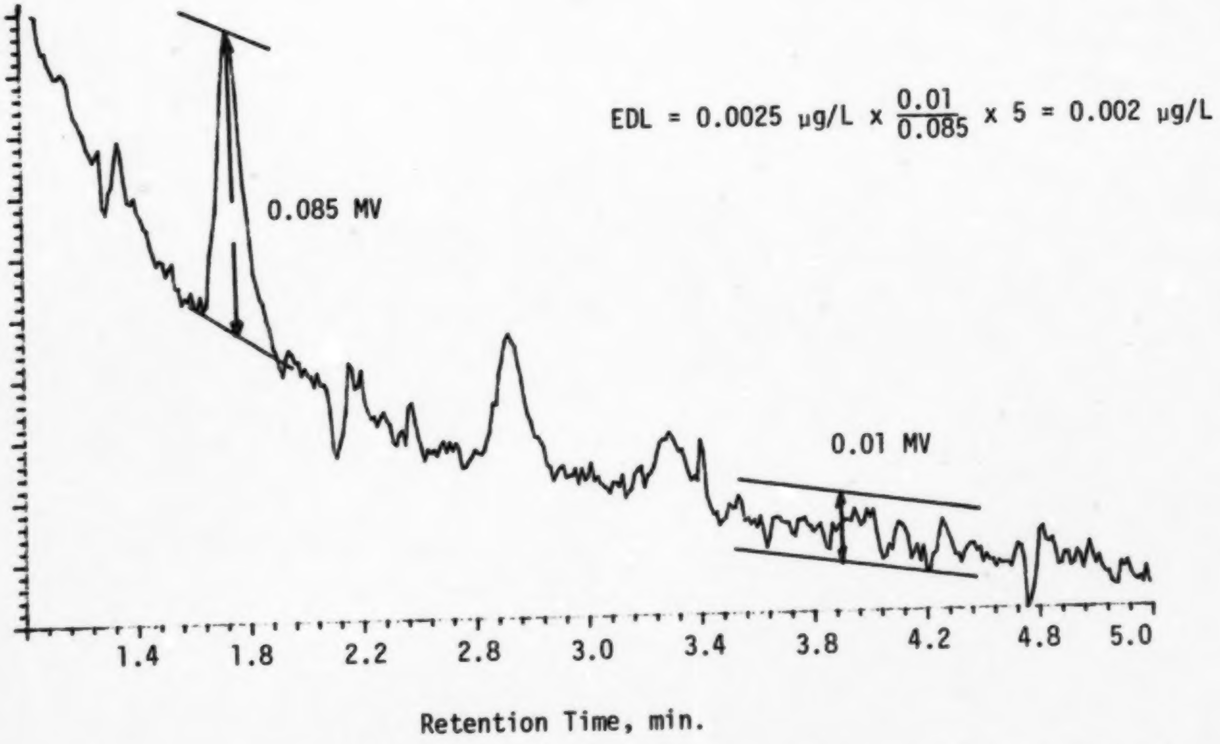
$$EDL = 0.025\ \mu g/L \times \frac{0.008}{0.066} \times 5 = 0.001\ \mu g/L$$

FIGURE 52. GC-ECD CHROMATOGRAM OF CHLOROTHALONIL STANDARD EQUIVALENT TO 0.0025 µg/L IN WATER USED TO DETERMINE METHOD EDL.



$$EDL = 0.0025\ \mu g/L \times \frac{0.013}{0.058} \times 5 = 0.003\ \mu g/L$$

FIGURE 53. GC-ECD CHROMATOGRAM OF DCPA STANDARD EQUIVALENT TO 0.0025 µg/L IN WATER USED TO DETERMINE METHOD EDL.



$$EDL = 0.0025\ \mu g/L \times \frac{0.01}{0.085} \times 5 = 0.002\ \mu g/L$$

FIGURE 54. GC-ECD CHROMATOGRAM OF DICLORAN STANDARD EQUIVALENT TO 0.0025 μg/L IN WATER USED TO DETERMINE METHOD EDL.

7.6. The EDL was calculated to be 0.002 µg/L of dicloran. The EDL for methoxychlor was determined by analyzing a 5 µg/mL solution of methoxychlor in hexane; the resultant chromatogram is shown in Figure 55. The methoxychlor solution yielded a S/N of 6.9. The EDL was calculated to be 0.04 µg/L of methoxychlor. The EDLs for cis-permethrin and trans-permethrin were determined by analyzing 10 µg/L standard solutions of these compounds in hexane; the resultant chromatograms are shown in Figures 56-57. The cis-permethrin solution yielded a S/N of 3.2. The EDL was calculated to be 0.2 µg/L of cis-permethrin. The trans-permethrin solution yielded a S/N of 2.2. The EDL was calculated to be 0.2 µg/L of trans-permethrin. The EDL does not take into account any interferences or recovery losses that might be encountered from a particular matrix.

Average peak areas from the analyses of standards of the organochlorine pesticides obtained from the chemical supply house did not vary by more than ±10 percent with the exception of cis-permethrin. The peak areas obtained from the chemical supply house souce of cis-permethrin were 40 percent lower than those obtained from the reference standards repository. It can only be assumed that the standard obtained from the chemical supply house was not as pure as was specified by the supplier.

RECOMMENDATIONS

The method is acceptable for the determination of cis-permethrin and trans-permethrin in water. The method was not found to be suitable for determination of chlorothalonil and dicloran in water samples. It was not possible to determine recoveries of chlorothalonil and dicloran from water due to the presence of interfering peaks. It is not unusual to have problems with interferences when an electron capture detector is used. This problem is magnified when the compounds of interest elute early, since chlorine-containing low molecular weight compounds are often present in water samples or introduced during processing of samples in the laboratory. It may be necessary to modify GC parameters or to use another GC column to change the chromatography of these two compounds. It may also be necessary to use another detector that is chlorine or nitrogen specific, such as a Hall detector in the halogen mode or a nitrogen-phosphorus detector to reduce the interference problem. Recoveries of DCPA and methoxychlor were concentration and/or matrix dependent. Other extraction methods such as continuous extraction or tumbling should be investigated to ascertain if the recoveries of DCPA or methoxychlor from water can be improved. The amount of mixing achieved between aqueous and organic phases using separatory funnels is operator dependent. Use of continuous extractors or tumbling assures optimum equilibrium conditions.

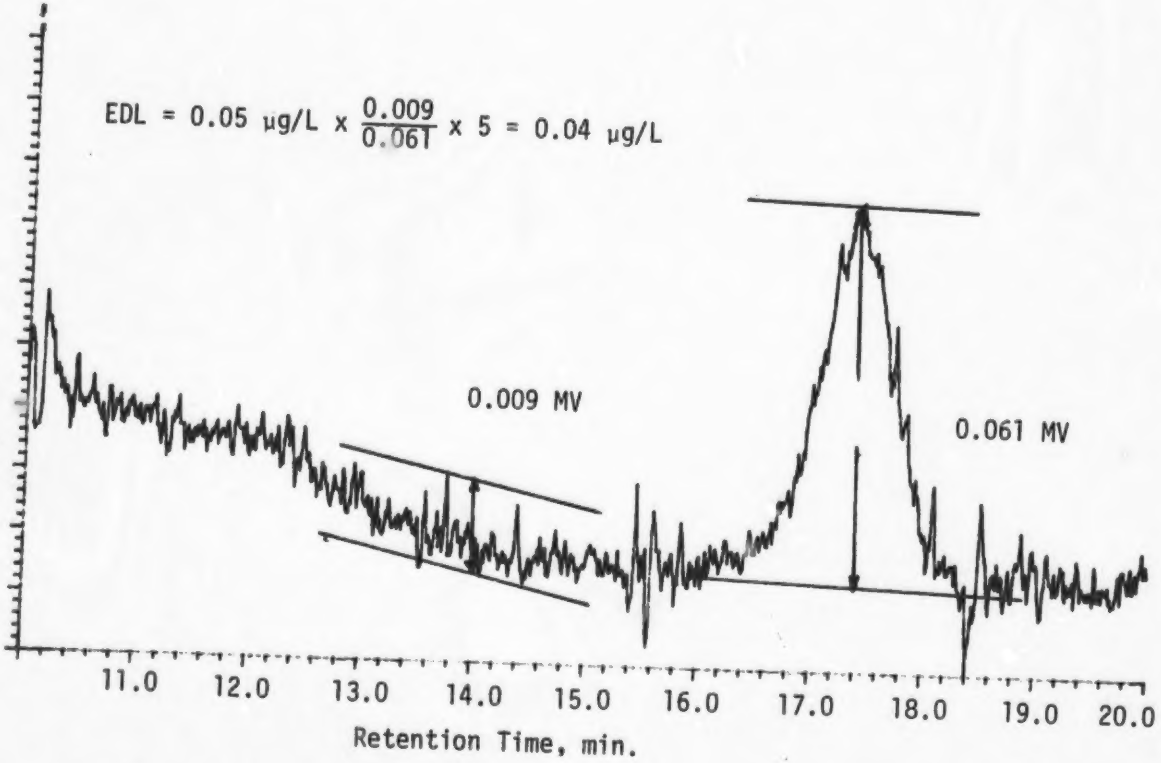


FIGURE 55. GC-ECD CHROMATOGRAM OF METHOXYCHLOR STANDARD EQUIVALENT TO 0.05 µg/L IN WATER USED TO DETERMINE METHOD EDL.

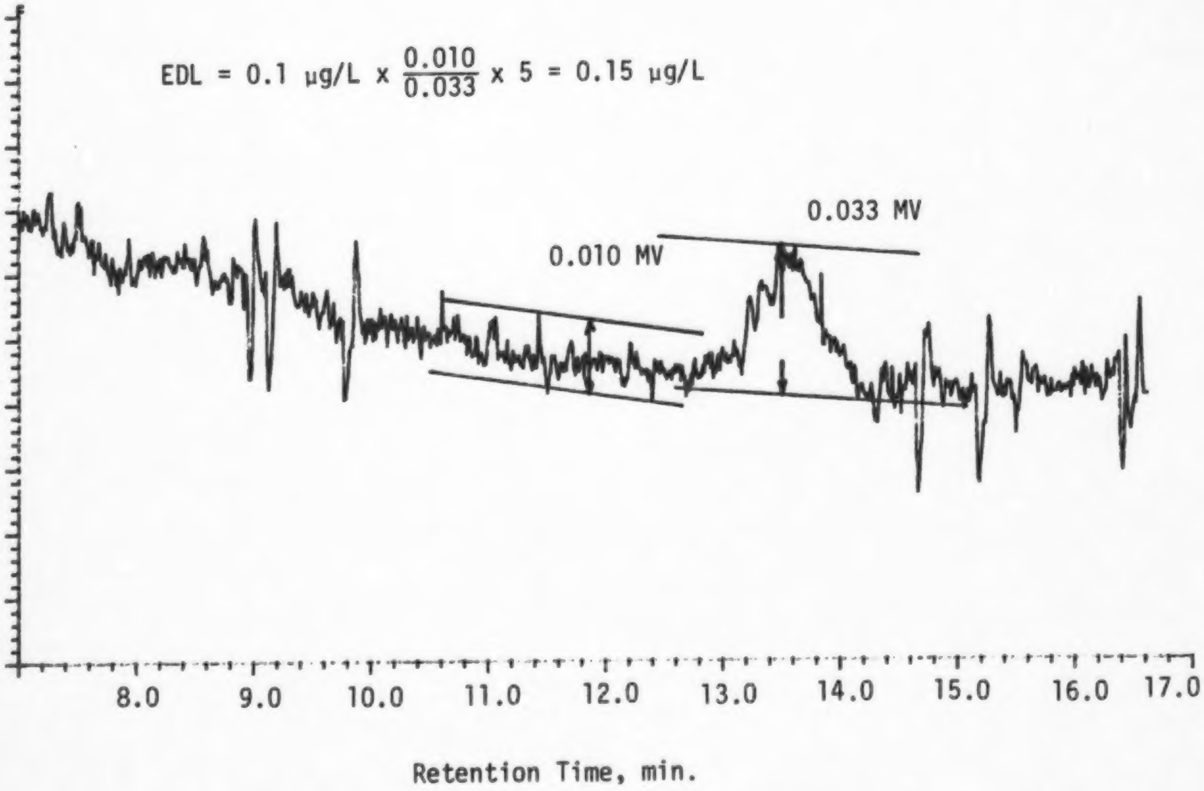


FIGURE 56. GC-ECD CHROMATOGRAM OF CIS-PERMETHRIN STANDARD EQUIVALENT TO 0.1 µg/L IN WATER USED TO DETERMINE METHOD EDL.

$$EDL = 0.1\ \mu g/L \times \frac{0.014}{0.030} \times 5 = 0.22\ \mu g/L$$

0.030 MV
0.014 MV
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0
16.0
17.0
Retention Time, min.

FIGURE 57. GC-ECD CHROMATOGRAM OF TRANS-PERMETHRIN STANDARD EQUIVALENT TO 0.1 μg/L IN WATER USED TO DETERMINE METHOD EDL.

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